1-F-6

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 385 962 B1

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent: **18.07.2001 Bulletin 2001/29**

(51) Int Cl.[7]: **C12N 15/82**, C12N 15/32, C12N 15/67, C12N 15/40, C12N 5/10, A01H 5/00

(21) Application number: **90870025.5**

(22) Date of filing: **20.02.1990**

(54) **Synthetic plant genes and method for preparation**

Synthetische Pflanzengene und Verfahren zu ihrer Herstellung

Gènes synthétiques de plantes et méthode pour leur préparation

(84) Designated Contracting States:
GR

(30) Priority: **24.02.1989 US 315355**
12.02.1990 US 476661

(43) Date of publication of application:
05.09.1990 Bulletin 1990/36

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Description

BACKGROUND OF THE INVENTION

[0001] The present invention relates to genetic engineering and more particularly to plant transformation in which a plant is transformed to express a heterologous gene.

[0002] Although great progress has been made in recent years with respect to transgenic plants which express foreign proteins such as herbicide resistant enzymes and viral coat proteins, very little is known about the major factors affecting expression of foreign genes in plants. Several potential factors could be responsible in varying degrees for the level of protein expression from a particular coding sequence. The level of a particular mRNA in the cell is certainly a critical factor.

[0003] The potential causes of low steady state levels of mRNA due to the nature of the coding sequence are many. First, full length RNA synthesis might not occur at a high frequency. This could, for example, be caused by the premature termination of RNA during transcription or due to unexpected mRNA processing during transcription. Second, full length RNA could be produced but then processed (splicing, polyA addition) in the nucleus in a fashion that creates a non-functional mRNA. If the RNA is properly synthesized, terminated and polyadenylated, it then can move to the cytoplasm for translation. In the cytoplasm, mRNAs have distinct half lives that are determined by their sequences and by the cell type in which they are expressed. Some RNAs are very short-lived and some are much more long-lived. In addtion, there is an effect, whose magnitude is uncertain, of translational efficiency on mRNA half-life. In addition, every RNA molecule folds into a particular structure, or perhaps family of sturctures, which is determined by its sequence. The particular structure of any RNA might lead to greater or lesser stability in the cytoplasm. Structure per se is probably also a determinant of mRNA processing in the nucleus. Unfortunately, it is impossible to predict, and nearly impossible to determine, the structure of any RNA (except for tRNA) in vitro or in vivo. However, it is likely that dramatically changing the sequence of an RNA will have a large effect on its folded structure. It is likely that structure per se or particular structural features also have a role in determining RNA stability.

[0004] Some particular sequences and signals have been identified in RNAs that have the potential for having a specific effect on RNA stability. This section summarizes what is known about these sequences and signals. These identified sequences often are A+T rich, and thus are more likely to occur in an A+T rich coding sequence such as a *B.t.* gene. The sequence motif ATTTA (or AUUUA as it appears in RNA) has been implicated as a destabilizing sequence in mammalian cell mRNA (Shaw and Kamen, 1986). No analysis of the function of this sequence in plants has been done. Many short lived mRNAs have A+T rich 3' untranslated regions, and these regions often have the ATTTA sequence, sometimes present in mutiple copies or as multimers (e.g., ATTTATTTA...). Shaw and Kamen showed that the transfer of the 3' end of an unstable mRNA to a stable RNA (globin or VA1) decreased the stable RNA's half life dramatically. They further showed that a pentamer of ATTTA had a profound destabilizing effect on a stable message, and that this signal could exert its effect whether it was located at the 3' end or within the coding sequence. However, the number of ATTTA sequences and/or the sequence context in which they occur also appear to be important in determining whether they function as destabilizing sequences. Shaw and Kamen showed that a trimer of ATTTA had much less effect than a pentamer on mRNA stability and a dimer or a monomer had no effect on stability (Shaw and Kamen, 1987). Note that multimers of ATTTA such as a pentamer automatically create an A+T rich region. This was shown to be a cytoplasmic effect, not nuclear. In other unstable mRNAs, the ATTTA sequence may be present in only a single copy, but it is often contained in an A+T rich region. From the animal cell data collected to date, it appears that ATTTA at least in some contexts is important in stability, but it is not yet possible to predict which occurences of ATTTA are destabiling elements or whether any of these effects are likely to be seen in plants.

[0005] Some studies on mRNA degradation in animal cells also indicate that RNA degradation may begin in some cases with nucleolytic attack in A+T rich regions. It is not clear if these cleavages occur at ATTTA sequences. There are also examples of mRNAs that have differential stability depending on the cell type in which they are expressed or on the stage within the cell cycle at which they are expressed. For example, histone mRNAs are stable during DNA synthesis but unstable if DNA synthesis is disrupted. The 3' end of some histone mRNAs seems to be responsible for this effect (Pandey and Marzluff, 1987). It does not appear to be mediated by ATTTA, nor is it clear what controls the differential stability of this mRNA. Another example is the differential stability of IgG mRNA in B lymphocytes during B cell maturation (Genovese and Milcarek, 1988). A final example is the instability of a mutant beta-thallesemic globin mRNA. In bone marrow cells, where this gene is normally expressed, the mutant mRNA is unstable, while the wild-type mRNA is stable. When the mutant gene is expressed in HeLa or L cells in vitro, the mutant mRNA shows no instability (Lim et al., 1988). These examples all provide evidence that mRNA stability can be mediated by cell type or cell cycle specific factors. Furthermore this type of instability is not yet associated with specific sequences. Given these uncertainties, it is not possible to predict which RNAs are likely to be unstable in a given cell. In addition, even the ATTTA motif may act differentially depending on the nature of the cell in which the RNA is present. Shaw and Kamen (1987) have reported that activation of protein kinase C can block degradation mediated by ATTTA.

[0006] The addition of a polyadenylate string to the 3' end is common to most eucaryotic mRNAs, both plant and animal. The currently accepted view of polyA addition is that the nascent transcript extends beyond the mature 3' terminus. Contained within this transcript are signals for polyadenylation and proper 3' end formation. This processing at the 3' end involves cleavage of the mRNA and addition of polyA to the mature 3' end. By searching for consensus sequences near the polyA tract in both plant and animal mRNAs, it has been possible to identify consensus sequences that apparently are involved in polyA addition and 3' end cleavage. The same consensus sequences seem to be important to both of these processes. These signals are typically a variation on the sequence AATAAA. In animal cells, some variants of this sequence that are functional have been identified; in plant cells there seems to be an extended range of functional sequences (Wickens and Stephenson, 1984; Dean et al., 1986). Because all of these consensus sequences are variations on AATAAA, they all are A+T rich sequences. This sequence is typically found 15 to 20 bp before the polyA tract in a mature mRNA. Experiments in animal cells indicate that this sequence is involved in both polyA addition and 3' maturation. Site directed mutations in this sequence can disrupt these functions (Conway and Wickens, 1988; Wickens et al., 1987). However, it has also been observed that sequences up to 50 to 100 bp 3' to the putative polyA signal are also required; i.e., a gene that has a normal AATAAA but has been replaced or disrupted downstream does not get properly polyadenylated (Gil and Proudfoot, 1984; Sadofsky and Alwine, 1984; McDevitt et al., 1984). That is, the polyA signal itself is not sufficient for complete and proper processing. It is not yet known what specific downstream sequences are required in addition to the polyA signal, or if there is a specific sequence that has this function. Therefore, sequence analysis can only identify potential polyA signals.

[0007] In naturally occuring mRNAs that are normally polyadenylated, it has been observed that disruption of this process, either by altering the polyA signal or other sequences in the mRNA, profound effects can be obtained in the level of functional mRNA. This has been observed in several naturally occuring mRNAs, with results that are gene specific so far. There are no general rules that can be derived yet from the study of mutants of these natural genes, and no rules that can be applied to heterologous genes. Below are four examples:

1. In a globin gene, absence of a proper polyA site leads to improper termination of transcription. It is likely, but not proven, that the improperly terminated RNA is nonfunctional and unstable (Proudfoot et al., 1987).
2. In a globin gene, absence of a functional polyA signal can lead to a 100-fold decrease in the level of mRNA accumulation (Proudfoot et al., 1987).
3. A globin gene polyA site was placed into the 3' ends of two different histone genes. The histone genes contain a secondary structure (stem-loop) near their 3' ends. The amount of properly polyadenylated histone mRNA produced from these chimeras decreased as the distance between the stem-loop and the polyA site increased. Also, the two histone genes produced greatly different levels of properly polyadenylated mRNA. This suggests an interaction between the polyA site and other sequences on the mRNA that can modulate mRNA accumulation (Pandy and Marzluff, 1987).
4. The soybean leghemoglobin gene has been cloned into HeLa cells, and it has been determined that this plant gene contains a "cryptic" polyadenylation signal that is active in animal cells, but is not utilized in plant cells. This leads to the production of a new polyadenylated mRNA that is nonfunctional. This again shows that analysis of a gene in one cell type cannot predict its behavior in alternative cell types (Wiebauer et al., 1988).

[0008] From these examples, it is clear that in natural mRNAs proper polyadenylation is important in mRNA accumulation, and that disruption of this process can effect mRNA levels significantly. However, insufficient knowledge exists to predict the effect of changes in a normal gene. In a heterologous gene, where we do not know if the putative polyA sites (consensus sequences) are functional, it is even harder to predict the consequences. However, it is possible that the putative sites identified are disfunctional. That is, these sites may not act as proper polyA sites, but instead function as aberrant sites that give rise to unstable mRNAs.

[0009] In animal cell systems, AATAAA is by far the most common signal identified in mRNAs upstream of the polyA, but at least four variants have also been found (Wickens and Stephenson, 1984). In plants, not nearly so much analysis has been done, but it is clear that multiple sequences similar to AATAAA can be used. The plant sites below called major or minor refer only to the study of Dean et al. (1986) which analyzed only three types of plant gene. The designation of polyadenylation sites as major or minor refers only to the frequency of their occurrence as functional sites in naturally occurring genes that have been analyzed. In the case of plants this is a very limited database. It is hard to predict with any certainty that a site designated major or minor is more or less likely to function partially or completely when found in a heterologous gene such as *B.t.*

PA	AATAAA	Major consensus site
P1A	AATAAT	Major plant site
P2A	AACCAA	Minor plant site
P3A	ATATAA	"
P4A	AATCAA	"
P5A	ATACTA	"
P6A	ATAAAA	"
P7A	ATGAAA	"
P8A	AAGCAT	"
P9A	ATTAAT	"
P10A	ATACAT	"
P11A	AAAATA	"

P12A	ATTAAA	Minor animal site
P13A	AATTAA	"
P14A	AATACA	"
P15A	CATAAA	"

[0010] Another type of RNA processing that occurs in the nucleus is intron splicing. Nearly all of the work on intron processing has been done in animal cells, but some data is emerging from plants. Intron processing depends on proper 5' and 3' splice junction sequences. Consensus sequences for these junctions have been derived for both animal and plant mRNAs, but only a few nucleotides are known to be invariant. Therefore, it is hard to predict with any certainty whether a putative splice junction is functional or partially functional based solely on sequence analysis. In particular, the only invariant nucleotides are GT at the 5' end of the intron and AG at the 3' end of the intron. In plants, at every nearby position, either within the intron or in the exon flanking the intron, all four nucleotides can be found, although some positions show some nucleotide preference (Brown, 1986; Hanley and Schuler, 1988).

[0011] A plant intron has been moved from a patatin gene into a GUS gene. To do this, site directed mutagenesis was performed to introduce new restriction sites, and this mutagenesis changed several nucleotides in the intron and exon sequences flanking the GT and AG. This intron still functioned properly, indicating the importance of the GT and AG and the flexibility at other nucleotide positons. There are of course many occurences of GT and AG in all genes that do not function as intron splice junctions, so there must be some other sequence or structrual features that identify splice junctions. In plants, one such feature appears to be base composition per se. Wiebauer et al. (1988) and Goodall et al. (1988) have analyzed plant introns and exons and found that exons have ~50% A+T while introns have ~70% A+T. Goodall et al. (1988) also created an artificial plant intron that has consensus 5' and 3' splice junctions and a random A+T rich internal sequence. This intron was spliced correctly in plants. When the internal segment was replaced by a G+C rich sequence, splicing efficiency was drastically reduced. These two examples demonsatrate that intron recognition in plants may depend on very general features -- splice junctions that have a great deal of sequence diversity and A+T richness of the intron itself. This, of course, makes it difficult to predict from sequence alone whether any particular sequence is likely to function as an active or partially active intron for RNA processing.

[0012] *B.t.* genes being A+T rich contain numerous stretches of various lengths that have 70% or greater A+T. The number of such stretches identified by sequence analysis depends on the length of sequence scanned.

[0013] As for polyadenylation described above, there are complications in predicting what sequences might be utilized as splice sites in any given gene. First, many naturally occuring genes have alternative splicing pathways that create alternative combinations of exons in the final mRNA (Gallega and Nadal-Ginard, 1988; Helfman and Ricci, 1988; Tsurushita and Korn, 1989). That is, some splice junctions are apparently · recognized under some circumstances or

in certain cell types, but not in others. The rules governing this are not understood. In addition, there can be an interaction between processing paths such that utilization of a particular polyadenylation site can interfere with splicing at a nearby splice site and vice versa (Adami and Nevins, 1988; Brady and Wold, 1988; Marzluff and Pandey, 1988). Again no predictive rules are available. Also, sequence changes in a gene can drastically alter the utilization of particular splice junctions. For example, in a bovine growth hormone gene, small deletions in an exon a few hundred bases downstream of an intron cause the splicing efficiency of the intron to drop from greater than 95% to less than 2% (essentially nonfunctional). Other deletions however have essentially no effect (Hampson and Rottman, 1988). Finally, a variety of in vitro and in vivo experiments indicate that mutations that disrupt normal splicing lead to rapid degradation of the RNA in the nucleus. Splicing is a multistep process in the nucleus and mutations in normal splicing can lead to blockades in the process at a variety of steps. Any of these blockades can then lead to an abnormal and unstable RNA. Studies of mutants of normally processed (polyadenylation and splicing) genes are relevant to the study of heterologous genes such as *B.t. B.t.* genes might contain functional signals that lead to the production of aberrant nonfunctional mRNAs, and these mRNAs are likely to be unstable. But the *B.t.* genes are perhaps even more likely to contain signals that are analogous to mutant signals in a natural gene. As shown above these mutant signals are very likely to cause defects in the processing pathways whose consequence is to produce unstable mRNAs.

[0014] It is not known with any certainty what signals RNA transcription termination in plant or animal cells. Some studies on animal genes that indicate that stretches of sequence rich in T cause termination by calf thymus RNA polymerase II in vitro. These studies have shown that the 3' ends of in vitro terminated transcripts often lie within runs of T such as T5, T6 or T7. Other identified sites have not been composed solely of T, but have had one or more other nucleotides as well. Termination has been found to occur within the sequences TATTTTTT, ATTCTC, TTCTT (Dedrick et al., 1987; Reines et al., 1987). In the case of these latter two, the context in which the sequence is found has been C+T rich as well. It is not known if this is essential. Other studies have implicated stretches of A as potential transcriptional terminators. An interesting example from SV40 illustrates the uncertainty in defining terminators based on sequence alone. One potential terminator in SV40 was identified as being A rich and having a region of dyad symmetry (potential stem-loop) 5' to the A rich stretch. However, a second terminator identified experimentally downstream in the same gene was not A rich and included no potential secondary structure (Kessler et al., 1988). Of course, due to the A+T content of *B.t.* genes, they are rich in runs of A or T that could act as terminators. The importance of termination to stability of the mRNA is shown by the globin gene example described above. Absence of a normal polyA site leads to a failure in proper termination with a consequent decrease in mRNA.

[0015] There is also an effect on mRNA stability due the translation of the mRNA. Premature translational termination in human triose phosphate isomerase leads to instability of the mRNA (Daar et al., 1988). Another example is the beta-thallesemic globin mRNA described above that is specifically unstable in bone marrow cells (Lim et al., 1988). The defect in this mutant gene is a single base pair deletion at codon 44 that leads to translational termination (a nonsense codon) at codon 60. Compared to properly translated normal globin mRNA, this mutant RNA is very unstable. These results indicate that an improperly translated mRNA is unstable. Other work in yeast indicates that proper but poor translation can have an effect on mRNA levels. A heterologous gene was modified to convert certain codons to more yeast preferred codons. An overall 10-fold increase in protein production was achieved, but there was also about a 3-fold increase in mRNA Hoekema et al., 1987). This indicates that more efficient translation can lead to greater mRNA stability, and that the effect of codon usage can be at the RNA level as well as the translational level. It is not clear from codon usage studies which codons lead to poor translation, or how this is coupled to mRNA stability.

[0016] EP-A-0 359 472 discloses modifying B.t. sequences to render them more plant-like. The sequence is modified so that the codon usage in the sequence is approximately the same as the codon usage in a plant. In contrast, the claimed invention is related to a specific methodology for increasing the expression of the gene in a plant by removing the occurrence of particular DNA sequences.

[0017] Therefore, it is an object of the present invention to provide a method for preparing synthetic plant genes which express their respective proteins at relatively high levels when compared to wild-type genes. It is yet another object of the present invention to provide synthetic plant genes which express the crystal protein toxin of *Bacillus thuringiensis* at relatively high levels.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018]

Figure 1 illustrates the steps employed in modifying a wild-type gene to increase expression efficiency in plants.

Figure 2 illustrates a comparison of the changes in the modified *B.t.k.* HD-1 sequence of Example 1 (lower line) versus the wild-type sequence of *B.t.k.* HD-1 which encodes the crystal protein toxin (upper line).

Figure 3 illustrates a comparison of the changes in the synthetic *B.t.k.* HD-1 sequence of Example 2 (lower line) versus the wild-type sequence of *B.t.k.* HD-1 which encodes the crystal protein toxin (upper line).

Figure 4 illustrates a comparison of the changes in the synthetic *B.t.k.* HD-73 sequence of Example 3 (lower line) versus the wild-type sequence of *B.t.k.* HD-73 (upper line).
Figure 5 represents a plasmid map of intermediate plant transformation vector cassette pMON893.
Figure 6 represents a plasmid map of intermediate plant transformation vector cassette pMON900.
Figure 7 represents a map for the disarmed T-DNA of *A. tumefaciens* ACO.
Figure 8 illustrates a comparison of the changes in the synthetic truncated *B.t.k.* HD-73 gene (Amino acids 29-615 with an N-terminal Met-Ala) of Example 3 (lower line) versus the wild-type sequence of *B.t.k.* HD-73 (upper line).
Figure 9 illustrates a comparison of the changes in the synthetic/wild-type full length *B.t.k.* HD-73 sequence of Example 3 (lower line) versus the wild-type full-length sequence of *B.t.k.* HD-73 (upper line).
Figure 10 illustrates a comparison of the changes in the synthetic/modified full length *B.t.k.* HD-73 sequence of Example 3 (lower line) versus the wild-type full-length sequence of *B.t.k.* HD-73 (upper line).
Figure 11 illustrates a comparison of the changes in the fully synthetic full-length *B.t.k.* HD-73 sequence of Example 3 (lower line) versus the wild-type full-length sequence of *B.t.k.* HD-73 (upper line).
Figure 12 illustrates a comparison of the changes in the synthetic *B.t.t.* sequence of Example 5 (lower line) versus the wild-type sequence of *B.t.t.* which encodes the crystal protein toxin (upper line).
Figure 13 illustrates a comparison of the changes in the synthetic *B.t.* P2 sequence of Example 6 (lower
Figure 14 illustrates a comparison of the changes in the synthetic *B.t. entomocidus* sequence of Example 7 (lower line) versus the wild-type sequence of *B.t.* entomocidus which encodes the Btent protein toxin (upper line).
Figure 15 illustrates a plasmid map for plant expression cassette vector pMON744.
Figure 16 illustrates a comparison of the changes in the synthetic potato leaf roll virus (PLRV) coat protein sequence of Example 9 (lower line) versus the wild-type coat protein sequence of PLRV (upper line).

STATEMENT OF THE INVENTION

[0019] The present invention provides a method for modifying a wild-type structural gene sequence which encodes an insecticidal protein of *Bacillus thuringiensis* to enhance the expression of said protein in plants which comprises:

a) identifying regions within said sequence with greater than four consecutive adenine or thymine nucleotides;

b) modifying the regions of step (a) which have two or more polyadenylation signals within a ten base sequence to remove said signals while maintaining a gene sequence which encodes said protein; and

c) modifying the 15-30 base regions surrounding the regions of step (a) to remove major plant polyadenylation signals, consecutive sequences containing more than one minor polyadenylation signal and consecutive sequences containing more than one ATTTA sequence while maintaining a gene sequence which encodes said protein.

[0020] The invention further provides a method for modifying a wild-type structural gene sequence which encodes an insecticidal protein of *Bacillus thuringiensis* to enhance the expression of said protein in plants which comprises:

a) removing polyadenylation signals contained in said wild-type gene while retaining a sequence which encodes said protein; and

b) removing ATTTA sequences contained in said wild-type gene while retaining a sequence which encodes said protein.

[0021] According to a further embodiment a method for improving the expression of a heterologous gene in plants is provided, wherein said gene comprises a modified chimeric gene containing a promoter which functions in plant cells operably linked to a structural coding sequence and a 3' non-translated region containing a polyadenylation signal which functions in plants to cause the addition of polyadenylate nucleotides to the 3' end of the RNA, and wherein said structural coding sequence encodes an insecticidal protein at least a portion of which was derived from a *Bacillus thuringiensis* protein, wherein said method comprises modifying said structural coding sequence so that said sequence has a DNA sequence which differs from the naturally occurring DNA sequence encoding said *Bacillus thuringiensis* protein and said structural coding sequence does not contain more than 5 consecutive nucleotides consisting of either adenine or thymine residues.

[0022] As a further embodiment, a method for improving the expression of a heterologous gene in plants is provided, wherein said gene comprises a modified chimeric gene containing a promoter which functions in plant cells operably linked to a structural coding sequence and a 3' non-translated region containing a polyadenylation signal which functions in plants to cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural

coding sequence encodes an insecticidal protein at least a portion of which was derived from a *Bacillus thuringiensis* protein, wherein said method comprises modifying said structural coding sequence so that said sequence has a DNA sequence which differs from the naturally occurring DNA sequence encoding said *Bacillus thuringiensis* protein and has the following characteristics:

said structural coding sequence has a region which is complementary to the following sequence:

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GGCTTGATTCCTAGCGAACTCTTCGATTCTCTGGTTGATGAGCTGTTC
1   5    10   15   20   25   30   35   40   45
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said region in said coding sequence having eliminated 2 AACCAA and 1 AATTAA sequence.

[0023] The present invention provides a method for preparing synthetic plant genes which encode the crystal protein toxin of *Bacillus thuringiensis* (*B.t.*). Suitable *B.t.* subspecies include, but are not limited to, *B.t. kurstaki* HD-1, *B.t. kurstaki* HD-73, *B.t. sotto, B.t. berliner, B.t. thuringiensis, B.t. tolworthi, B.t. dendrolimus, B.t. alesti, B.t. galleriae, B.t. aizawai, B.t. subtoxicus*, *B.t. entomocidus*, *B.t. tenebrionis* and *B.t. san diego*.

[0024] The expression of *B.t.* genes in plants is problematic. Although the expression of *B.t.* genes in plants at insecticidal levels has been reported, this accomplishment has not been straightforward. In particular, the expression of a full-length lepidopteran specific *B.t.* gene (comprising DNA from a *B.t.k.* isolate) has been reported to be unsuccessful in yielding insecticidal levels of expression in some plant species (Vaeck et al., 1987 and Barton et al., 1987).

[0025] It has been reported that expression of the full-length gene from *B.t.k.* HD-1 was detectable in tomato plants but that truncated genes led to a higher frequency of insecticidal plants with an overall higher level of expression. Truncated genes of *B.t. berliner* also led to a higher frequency of insecticidal plants in tobacco (Vaeck et al., 1987). On the other hand, insecticidal plants were provided from lettuce transformants using a full-length gene.

[0026] It has also been reported that the full length gene from *B.t.k.* HD-73 gave some insecticidal effect in tobacco (Adang et al., 1987). However, the *B.t.* mRNA detected in these plants was only 1.7 kb compared to the expected 3.7 kb indicating improper expression of the gene. It was suggested that this truncated mRNA was too short to encode a functional truncated toxin, but there must have been a low level of longer mRNA in some plants or no insecticidal activity would have been observed. Others have reported in a publication that they observed a large amount of shorter than expected mRNA from a truncated *B.t.k.* gene, but some mRNA of the expected size was also observed. In fact, it was suggested that expression of the full length gene is toxic to tobacco callus (Barton et al., 1987). The above illustrates that lepidopteran type *B.t.* genes are poorly expressed in plants compared to other chimeric genes previously expressed from the same promoter cassettes.

[0027] The expression of *B.t.t.* in tomato and potato is at levels similar to that of *B.t.k.* (i.e., poor). *B.t.t.* and *B.t.k.* genes share only limited sequence homology, but they share many common features in terms of base composition and the presence of particular A+T rich elements.

[0028] All reports in the field have noted the lower than expected expression of *B.t.* genes in plants. In general, insecticidal efficacy has been measured using insects very sensitive to *B.t.* toxin such as tobacco hornworm. Although it has been possible to obtain plants totally protected against tobacco hornworm, it is important to note that hornworm is up to 500 fold more sensitive to *B.t.* toxin than some agronomically important insect pests such as beet armyworm. It is therefore of interest to obtain transgenic plants that are protected against all important lepidopteran pests (or against Colorado potato beetle in the case of *B.t. tenebrionis*), and in addition to have a level of *B.t.* expression that provides an additional safety margin over and above the efficacious protection level. It is also important to devise plant genes which function reproducibly from species to species, so that insect resistant plants can be obtained in a predictable fashion.

[0029] In order to achieve these goals, it is important to understand the nature of the poorer than expected expression of *B.t.* genes in plants. The level of stable *B.t.* mRNA in plants is much lower than expected. That is, compared to other coding sequences driven by the same promoter, the level of *B.t.* mRNA measured by Northern analysis or nuclease protection experiments is much lower. For example, tomato plant 337 (Fischhoff et al., 1987) was selected as the best expressing plant with pMON9711 which contains the *B.t.k.* HD-1 KpnI fragment driven by the CaMV 35S promoter and contains the NOS-NPTII-NOS selectable marker gene. In this plant the level of *B.t.* mRNA is between 100 to 1000 fold lower than the level of NPTII mRNA, even though the 35S promoter is approximately 50-fold stronger than the NOS promoter (Sanders et al., 1987).

[0030] The level of *B.t.* toxin protein detected in plants is consistent with the low level of *B.t.* mRNA. Moreover, the insecticidal efficacy of the transgenic plants correlates with the *B.t.* protein level indicating that the toxin protein produced in plants is biologically active. Therefore, the low level of *B.t.* toxin expression may be the result of the low levels

of *B.t.* mRNA.

[0031] Messenger RNA levels are determined by the rate of synthesis and rate of degradation. It is the balance between these two that determines the steady state level of mRNA. The rate of synthesis has been maximized by the use of the CaMV 35S promoter, a strong constitutive plant expressible promoter. The use of other plant promoters such as nopaline synthase (NOS), mannopine synthase (MAS) and ribulose bisphosphatecarboxylase small subunit (RUBISCO) have not led to dramatic changes in the levels of *B.t.* toxin protein expression indicating that the effects determining *B.t.* toxin protein levels are promoter independent. These data imply that the coding sequences of DNA genes encoding *B.t.* toxin proteins are somehow responsible for the poor expression level, and that this effect is manifested by a low level of accumulated stable mRNA.

[0032] Lower than expected levels of mRNA have been observed with four different lepidopteran specific genes (two from *B.t.k.* HD-1; *B.t. berliner* and *B.t.k.* HD-73) as well as the gene from the coleopteran specific *B.t. tenebrionis.* It appears that for lepidopteran type *B.t.* genes these effects are manifest more strongly in the full length coding sequences than in the truncated coding sequences. These effects are seen across plant species although their magnitude seems greater in some plant species such as tobacco.

[0033] The nature of the coding sequences of *B.t.* genes distinguishes them from plant genes as well as many other heterologous genes expressed in plants. In particular, *B.t.* genes are very rich (~62%) in adenine (A) and thymine (T) while plant genes and most bacterial genes which have been expressed in plants are on the order of 45-55% A+T. The A+T content of the genomes (and thus the genes) of any organism are features of that organism and reflect its evolutionary history. While within any one organism genes have similar A+T content, the A+T content can vary tremendously from organism to organism. For example, some *Bacillus* species have among the most A+T rich genomes while some *Steptomyces* species are among the least A+T rich genomes (~30 to 35% A+T).

[0034] Due to the degeneracy of the genetic code and the limited number of codon choices for any amino acid, most of the "excess" A+T of the structural coding sequences of some *Bacillus* species are found in the third position of the codons. That is, genes of some *Bacillus* species have A or T as the third nucleotide in many codons. Thus A+T content in part can determine codon usage bias. In addition, it is clear that genes evolve for maximum function in the organism in which they evolve. This means that particular nucleotide sequences found in a gene from one organism, where they may play no role except to code for a particular stretch of amino acids, have the potential to be recognized as gene control elements in another organism (such as transcriptional promoters or terminators, polyA addition sites, intron splice sites, or specific mRNA degradation signals). It is perhaps surprising that such misread signals are not a more common feature of heterologous gene expression, but this can be explained in part by the relatively homogeneous A+T content (~50%) of many organisms. This A+T content plus the nature of the genetic code put clear constraints on the likliehood of occurence of any particular oligonucleotide sequence. Thus, a gene from *E. coli* with a 50% A+T content is much less likely to contain any particular A+T rich segment than a gene from *B. thuringiensis.*

[0035] As described above, the expression of *B.t.* toxin protein in plants has been problematic. Although the observations made in other systems described above offer the hope of a means to elevate the expression level of *B.t.* toxin proteins in plants, the success obtained by the present method is quite unexpected. Indeed, inasmuch as it has been recently reported that expression of the full-length *B.t.k.* toxin protein in tobacco makes callus tissue necrotic (Barton et al., 1987); one would reasonably expect that high level expression of *B.t.* toxin protein to be unattainable due to the reported toxicity effects.

[0036] In its most rigorous application, the method of the present invention involves the modification of an existing structural coding sequence ("structural gene") which codes for a particular protein by removal of ATTTA sequences and putative polyadenylation signals by site directed mutagenesis of the DNA comprising the structural gene. It is most preferred that substantially all the polyadenylation signals and ATTTA sequences are removed although enhanced expression levels are observed with only partial removal of either of the above identified sequences. Alternately if a synthetic gene is prepared which codes for the expression of the subject protein, codons are selected to avoid the ATTTA sequence and putative polyadenylation signals. For purposes of the present invention putative polyadenylation signals include, but are not necessarily limited to, AATAAA, AATAAT, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA and CATAAA. In replacing the ATTTA sequences and polyadenylation signals, codons are preferably utilized which avoid the codons which are rarely found in plant genomes.

[0037] Another embodiment of the present invention, represented in the flow diagram of Figure 1, employs a method for the modification of an existing structural gene or alternately the de *novo* synthesis of a structural gene which method is somewhat less rigorous than the method first described above. Referring to Figure 1, the selected DNA sequence is scanned to identify regions with greater than four consecutive adenine (A) or thymine (T) nucleotides. The A+T regions are scanned for potential plant polyadenylation signals. Although the absence of five or more consecutive A or T nucleotides eliminates most plant polyadenylation signals, if there are more than one of the minor polyadenylation signals identified within ten nucleotides of each other, then the nucleotide sequence of this region is preferably altered to remove these signals while maintaining the original encoded amino acid sequence.

[0038] The second step is to consider the 15 to 30 nucleotide regions surrounding the A+T rich region identified in step one. If the A+T content of the surrounding region is less than 80%, the region should be examined for polyadenylation signals. Alteration of the region based on polyadenylation signals is dependent upon (1) the number of polyadenylation signals present and (2) presence of a major plant polyadenylation signal.

[0039] The extended region is examined for the presence of plant polyadenylation signals. The polyadenylation signals are removed by site-directed mutagenesis of the DNA sequence. The extended region is also examined for multiple copies of the ATTTA sequence which are also removed by mutagenesis.

[0040] It is also preferred that regions comprising many consecutive A+T bases or G+C bases are disrupted since these regions are predicted to have a higher likelihood to form hairpin structure due to self-complementarity. Therefore, insertion of heterogeneous base pairs would reduce the likelihood of self-complementary secondary structure formation which are known to inhibit transcription and/or translation in some organisms. In most cases, the adverse effects may be minimized by using sequences which do not contain more than five consecutive A+T or G+C.

SYNTHETIC OLIGONUCLEOTIDES FOR MUTAGENESIS

[0041] The oligonucleotides used in the mutagenesis are designed to maintain the proper amino acid sequence and reading frame and preferably to not introduce common restriction sites such as BglII, HindIII, SacI, KpnI, EcoRI, NcoI, PstI and SalI into the modified gene. These restriction sites are found in multilinker insertion sites of cloning vectors such as plasmids pUC118 and pMON7258. Of course, the introduction of new polyadenylation signals, ATTTA sequences or consecutive stretches of more than five A+T or G+C, should also be avoided. The preferred size for the oligonucleotides is around 40-50 bases, but fragments ranging from 18 to 100 bases have been utilized. In most cases, a minimum of 5 to 8 base pairs of homology to the template DNA on both ends of the synthesized fragment are maintained to insure proper hybridization of the primer to the template. The oligonucleotides should avoid sequences longer than five base pairs A+T or G+C. Codons used in the replacement of wild-type codons should preferably avoid the TA or CG doublet wherever possible. Codons are selected from a plant preferred codon table (such as Table I below) so as to avoid codons which are rarely found in plant genomes, and efforts should be made to select codons to preferably adjust the G+C content to about 50%.

Table I

Preferred Codon Usage in Plants		
Amino Acid	Codon	Percent Usage in Plants
ARG	CGA	7
	CGC	11
	CGG	5
	CGU	25
	AGA	29
	AGG	23
LEU	CUA	8
	CUC	20
	CUG	10
	CUU	28
	UUA	5
	UUG	30
SER	UCA	14
	UCC	26
	UCG	3
	UCU	21
	AGC	21
	AGU	15
THR	ACA	21
	ACC	41

Table I (continued)

Preferred Codon Usage in Plants		
Amino Acid	Codon	Percent Usage in Plants
	ACG	7
	ACU	31
PRO	CCA	45
	CCC	19
	CCG	9
	CCU	26
ALA	GCA	23
	GCC	32
	GCG	3
	GCU	41
GLY	GGA	32
	GGC	20
	GGG	11
	GGU	37
ILE	AUA	12
	AUC	45
	AUU	43
VAL	GUA	9
	GUC	20
	GUG	28
	GUU	43
LYS	AAA	36
	AAG	64
ASN	AAC	72
	AAU	28
GLN	CAA	64
	CAG	36
HIS	CAC	65
	CAU	35
GLU	GAA	48
	GAG	52
ASP	GAC	48
	GAU	52
TYR	UAC	68
	UAU	32
CYS	UGC	78

Table I (continued)

Preferred Codon Usage in Plants		
Amino Acid	Codon	Percent Usage in Plants
	UGU	22
PHE	UUC	56
	UUU	44
MET	AUG	100
TRP	UGG	100

[0042] Regions with many consecutive A+T bases or G+C bases are predicted to have a higher likelihood to form hairpin structures due to self-complementarity. Disruption of these regions by the insertion of heterogeneous base pairs is preferred and should reduce the likelihood of the formation of self-complementary secondary structures such as hairpins which are known in some organisms to inhibit transcription (transcriptional terminators) and translation (attenuators). However, it is difficult to predict the biological effect of a potential hairpin forming region.

[0043] It is evident to those skilled in the art that while the above description is directed toward the modification of the DNA sequences of wild-type genes, the present method can be used to construct a completely synthetic gene for a given amino acid sequence. Regions with five or more consecutive A+T or G+C nucleotides should be avoided. Codons should be selected avoiding the TA and CG doublets in codons whenever possible. Codon usage can be normalized against a plant preferred codon usage table (such as Table I) and the G+C content preferably adjusted to about 50%. The resulting sequence should be examined to ensure that there are minimal putative plant polyadenylation signals and ATTTA sequences. Restriction sites found in commonly used cloning vectors are also' preferably avoided. However, placement of several unique restriction sites throughout the gene is useful for analysis of gene expression or construction of gene variants.

Plant Gene Construction

[0044] The expression of a plant gene which exists in double-stranded DNA form involves transcription of messenger RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3' non-translated region which adds polyadenylate nucleotides to the 3' end of the RNA. Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter." The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA and to initiate the transcription of mRNA using one of the DNA strands as a template to make a corresponding strand of RNA.

[0045] A number of promoters which are active in plant cells have been described in the literature. These include the nopaline synthase (NOS) and octopine synthase (OCS) promoters (which are carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the Cauliflower Mosaic Virus (CaMV) 19S and 35S promoters, the light-inducible promoter from the small subunit of ribulose bis-phosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide) and the mannopine synthase (MAS) promoter (Velten et al. 1984 and Velten & Schell, 1985). All of these promoters have been used to create various types of DNA constructs which have been expressed in plants (see e.g., PCT publication WO84/02913 (Rogers et al., Monsanto).

[0046] Promoters which are known or are found to cause transcription of RNA in plant cells can be used in the present invention. Such promoters may be obtained from plants or plant viruses and include, but are not limited to, the CaMV35S promoter and promoters isolated from plant genes such as ssRUBISCO genes. As described below, it is preferred that the particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of protein.

[0047] The promoters used in the DNA constructs (i.e. chimeric plant genes) of the present invention may be modified, if desired, to affect their control characteristics. For example, the CaMV35S promoter may be ligated to the portion of the ssRUBISCO gene that represses the expression of ssRUBISCO in the absence of light, to create a promoter which is active in leaves but not in roots. The resulting chimeric promoter may be used as described herein. For purposes of this description, the phrase "CaMV35S" promoter thus includes variations of CaMV35S promoter, e.g., promoters derived by means of ligation with operator regions, random or controlled mutagenesis, etc. Furthermore, the promoters may be altered to contain multiple "enhancer sequences" to assist in elevating gene expression.

[0048] The RNA produced by a DNA construct of the present invention also contains a 5' non-translated leader

sequence. This sequence can be derived from the promoter selected to express the gene, and can be specifically modified so as to increase translation of the mRNA. The 5' non-translated regions can also be obtained from viral RNA's, from suitable eukaryotic genes, or from a synthetic gene sequence. The present invention is not limited to constructs, as presented in the following examples. Rather, the non-translated leader sequence can be part of the 5' end of the non-translated region of the coding sequence for the virus coat protein, or part of the promoter sequence, or can be derived from an unrelated promoter or coding sequence. In any case, it is preferred that the sequence flanking the initiation site conform to the translational consensus sequence rules for enhanced translation initiation reported by Kozak (1984).

[0049] The DNA construct of the present invention also contains a modified or fully-synthetic structural coding sequence encoding the crystal toxin protein of *Bacillus thuringiensis* which has been changed to enhance the performance of the gene in plants. The structural genes of the present invention may optionally encode a fusion protein comprising an amino-terminal chloroplast transit peptide or secretory signal sequence (see for instance, Examples 10 and 11).

[0050] The DNA construct also contains a 3' non-translated region. The 3' non-translated region contains a polyadenylation signal which functions in plants to cause the addition of polyadenylate nucleotides to the 3' end of the viral RNA. Examples of suitable 3' regions are (1) the 3' transcribed, non-translated regions containing the polyadenylation signal of *Agrobacterium* tumor-inducing (Ti) plasmid genes, such as the nopaline synthase (NOS) gene, and (2) plant genes like the soybean storage protein (7S) genes and the small subunit of the RuBP carboxylase (E9) gene. An example of a preferred 3' region is that from the 7S gene, described in greater detail in the examples below.

Plant Transformation

[0051] A chimeric plant gene containing a structural coding sequence of the present invention can be inserted into the genome of a plant by any suitable method. Suitable plants for use in the practice of the present invention include, but are not limited to, soybean, cotton, alfalfa, oilseed rape, flax, tomato, sugarbeet, sunflower, potato, tobacco, maize, rice and wheat. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed, e.g., by Herrera-Estrella (1983), Bevan (1983), Klee (1985) and EPO publication 120,516 (Schilperoort et al.). In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used to insert the DNA constructs of this invention into plant cells. Such methods may involve, for example, the use of liposomes, electroporation, chemicals that increase free DNA uptake, free DNA delivery via microprojectile bombardment, and transformation using viruses or pollen.

[0052] A particularly useful Ti plasmid cassette vector for transformation of dicotyledonous plants is shown in Figure 5. Referring to Figure 5, the expression cassette pMON893 consists of the enhanced CaMV35S promoter (EN 35S) and the 3' end including polyadenylation signals from a soybean gene encoding the alpha-prime subunit of beta-conglycinin. Between these two elements is a multilinker containing multiple restriction sites for the insertion of genes.

[0053] The enhanced CaMV35S promoter was constructed as follows. A fragment of the CaMV35S promoter extending between position -343 and +9 was previously constructed in pUC13 by Odell et al. (1985). This segment contains a region identified by Odell et al. (1985) as being necessary for maximal expression of the CaMV35S promoter. It was excised as a ClaI-HindIII fragment, made blunt ended with DNA polymerase I (Klenow fragment) and inserted into the HincII site of pUC18. This upstream region of the 35S promoter was excised from this plasmid as a HindIII-EcoRV fragment (extending from -343 to -90) and inserted into the same plasmid between the HindIII and PstI sites. The enhanced CaMV35S promoter thus contains a duplication of sequences between -343 and -90 (Kay et al., 1987).

[0054] The 3' end of the 7S gene is derived from the 7S gene contained on the clone designated 17.1 (Schuler et al., 1982). This 3' end fragment, which includes the polyadenylation signals, extends from an AvaII site located about 30 bp upstream of the termination codon for the beta-conglycinin gene in clone 17.1 to an EcoRI site located about 450 bp downstream of this termination codon.

[0055] The remainder of pMON893 contains a segment of pBR322 which provides an origin of replication in *E. coli* and a region for homologous recombination with the disarmed T-DNA in *Agrobacterium* strain ACO (described below); the oriV region from the broad host range plasmid RK1; the streptomycin/spectinomycin resistance gene from Tn7; and a chimeric NPTII gene, containing the CaMV35S promoter and the nopaline synthase (NOS) 3' end, which provides kanamycin resistance in transformed plant cells.

[0056] Referring to Figure 6, transformation vector plasmid pMON900 is a derivative of pMON893. The enhanced CaMV35S promoter of pMON893 has been replaced with the 1.5kb mannopine synthase (MAS) promoter (Velten et al. 1984). The other segments are the same as plasmid pMON893. After incorporation of a DNA construct into plasmid vector pMON893 or pMON900, the intermediate vector is introduced into A. tumefaciens strain ACO which contains a disarmed Ti plasmid. Cointegrate Ti plasmid vectors are selected and used to transform dicotyledonous plants.

[0057] Referring to Figure 7, A. *tumefaciens* ACO is a disarmed strain similar to pTiB6SE described by Fraley et al. (1985). For construction of ACO the starting *Agrobacterium* strain was the strain A208 which contains a nopaline-type Ti plasmid. The Ti plasmid was disarmed in a manner similar to that described by Fraley et al. (1985) so that essentially

all of the native T-DNA was removed except for the left border and a few hundred base pairs of T-DNA inside the left border. The remainder of the T-DNA extending to a point just beyond the right border was replaced with a novel piece of DNA including (from left to right) a segment of pBR322, the oriV region from plasmid RK2, and the kanamycin resistance gene from Tn601. The pBR322 and oriV segments are similar to the segments in pMON893 and provide a region of homology for cointegrate formation.

[0058] The following examples are provided to better elucidate the practice of the present invention and should not be interpreted in any way to limit the scope of the present invention. Those skilled in the art will recognize that various modifications, truncations etc. can be made to the methods and genes described herein while not departing from the spirit and scope of the present invention.

Example 1 – Modified *B.t.k.* HD-1 Gene

[0059] Referring to Figure 2, the wild-type *B.t.k.* HD-1 gene is known to be expressed poorly in plants as a full length gene or as a truncated gene. The G+C content of the *B.t.k.* gene is low (37%) containing many A+T rich regions, potential polyadenylation sites (18 sites; see Table II for the list of sequences) and numerous ATTTA sequences.

Table II

List of Sequences of the Potential Polyadenylation Signals

AATAAA*	AAGCAT
AATAAT*	ATTAAT
AACCAA	ATACAT
ATATAA	AAAATA
AATCAA	ATTAAA**
ATACTA	AATTAA**
ATAAAA	AATACA**
ATGAAA	CATAAA**

* indicates a potential major plant polyadenylation site.

** indicates a potential minor animal polyadenylation site.

All others are potential minor plant polyadenylation sites.

[0060] Table III lists the synthetic oligonucleotides designed and synthesized for the site-directed mutagenesis of the *B.t.k.* HD-1 gene.

Table III

Mutagenesis Primers for *B.t.k.* HD-1 Gene

Primer	Length (bp)	Sequence
BTK185	18	TCCCCAGATA ATATCAAC
BTK240	48	GGCTTGATTC CTAGCGAACT CTTCGATTCT CTGGTTGATG AGCTGTTC
BTK462	54	CAAAACTGAG AGGTGGAGGT TGGCAGCTTG AACGTACACG GAGAGGAGAGGAAC
BTK669	48	AGTTAGTGTA AGCTCTCTTC TGAACTGGTT GTACCTGATC CAATCTCT
BTK930	39	AGCCATGATC TGGTGACCGG ACCAGTAGTA TTCTCCTCT
BTK1110	32	AGTTGTTGGT TGTTGATCCC GATGTTAAAA GG

Table III - continued

Mutagenesis Primers for *B.t.k.* HD-1 Gene

Primer	Length (bp)	Sequence
BTK1380A	37	GTGATGAAGG GATGATGTTG TTGAACTCAG CACTACG
BTK1380T	100	CAGAAGTTCC AGAGCCAAGA TTAGTAGACT TGGTGAGTGG GATTTGGGTG ATTTGTGATG AAGGGATGAT GTTGTTGAAC TCAGCACTAC GATGTATCCA
BTK1600	27	TGATGTGTGG AACTGAAGGT TTGTGGT

[0061] The *B.t.k.* HD-1 gene (BglII fragment from pMON9921 encoding amino acids 29-607 with a Met-Ala at the N-terminus) was cloned into pMON7258 (pUC118 derivative which contains a BglII site in the multilinker cloning region) at the BglII site resulting in pMON5342. The orientation of the *B.t.k.* gene was chosen so that the opposite strand (negative strand) was synthesized in filamentous phage particles for the mutagenesis. The procedure of Kunkle (1985) was used for the mutagenesis using plasmid pMON5342 as starting material.

[0062] The regions for mutagenesis were selected in the following manner. All regions of the DNA sequence of the *B.t.k.* gene were identified which contained five or more consecutive base pairs which were A or T. These were ranked in terms of length and highest percentage of A+T in the surrounding sequence over a 20-30 base pair region. The DNA was then analysed for regions which might contain polyadenylation sites (see Table II above) or ATTTA sequences. Oligonucleotides were designed which maximized the elimination of A+T consecutive regions which contained one or more polyadenylation sites or ATTTA sequences. Two potential plant polyadenylation sites were rated more critical (see Table II) based on published reports. Codons were selected which increased G+C content, did not generate restriction sites for enzymes useful for cloning and assembly of the modified gene (BamHI, BglII, SacI, NcoI, EcoRV) and did not contain the doublets TA or GC which have been reported to be infrequently found in codons in plants. The oligonucleotides were at least 18 bp long ranging up to 100 base pairs and contained at least 5-8 base pairs of direct homology to native sequences at the ends of the fragments for efficient hybridization and priming in site-directed mutagenesis reactions. Figure 2 compares the wild-type *B.t.k.* HD-1 gene sequence with the sequence which resulted from the modifications by site-directed mutagenesis.

[0063] The end result of these changes was to increase the G+C content of *B.t.k.* gene from 37% to 41% while also decreasing the potential plant polyadenylation sites from 18 to 7 and decreasing the ATTTA regions from 13 to 7. Specifically, the mutagenesis changes from amino (5') terminus to the carboxy (3') terminus are as follows:

[0064] BTK185 is an 18-mer used to eliminate a plant polyadenylation site in the midst of a nine base pair region of A+T.

[0065] BTK240 is a 48-mer. Seven base pairs were changed by this oligonucleotide to eliminate three potential polyadenylation sites (2 AACCAA, 1 AATTAA). Another region close to the region altered by BTK240, starting at bp 312, had a high A+T content (13 of 15 base pairs) and an ATTTA region. However, it did not contain a potential polyadenylation site and its longest string of uninterrupted A+T was seven base pairs.

[0066] BTK462 is a 54-mer introducing 13 base pair changes. The first six changes were to reduce the A+T richness of the gene by replacing wild-type codons with codons containing G and C while avoiding the CG doublet. The next

seven changes made by BTK462 were used to eliminate an A+T rich region (13 of 14 base pairs were A or T) containing two ATTTA regions.

[0067] BTK669 is a 48-mer making nine individual base pair changes eliminating three possible polyadenylation sites (ATATAA, AATCAA, and AATTAA) and a single ATTTA site.

[0068] BTK930 is a 39-mer designed to increase the G+C content and to eliminate a potential polyadenylation site (AATAAT - a major site). This region did contain a nine base pair region of consecutive A+T sequence. One of the base pair changes was a G to A because a G at this position would have created a G+C rich region (CCGG(G)C). Since sequencing reactions indicate that there can be difficulties generating sequence through G+C consecutive bases, it was thought to be prudent to avoid generating potentially problematic regions even if they were problematic only in vitro.

[0069] BTK1110 is a 32-mer designed to introduce five changes in the wild-type gene. One potential site (AATAAT - a major site) was eliminated in the midst of an A+T rich region (19 of 22 base pairs).

[0070] BTK1380A and BTK1380T are responsible for 14 individual base pair changes. The first region (1380A) has 17 consecutive A+T base pairs. In this region is an ATTTA and a potential polyadenylation site (AATAAT). The 100-mer (1380T) contains all the changes dictated by 1380A. The large size of this primer was in part an experiment to determine if it was feasible to utilize large oligonucleotides for mutagenesis (over 60 bases in length). A second consideration was that the 100-mer was used to mutagenize a template which had previously been mutageneized by 1380A. The original primer ordered to mutagenize the region downstream and adjacent to 1380A did not anneal efficiently to the desired site as indicated by an inability to obtain clean sequence utilizing the primer. The large region of homology of 1380T did assure proper annealing. The extended size of 1380T was more of a convenience rather than a necessity. The second region adjacent to 1380A covered by 1380T has a high A+T content (22 of 29 bases are A or T).

[0071] BTK1600 is a 27-mer responsible for five individual base pair changes. An ATTTA region and a plant polyadenylation site were identified and the appropriate changes engineered.

[0072] A total of 62 bases were changed by site-directed mutagenesis. The G+C content increased by 55 base pairs, the potential polyadenylation sites were reduced from 18 to seven and the ATTTA sequences decreased from 13 to seven. The changes in the DNA sequence resulted in changes in 55 of the 579 codons in the truncated *B.t.k.* gene in pMON5342 (approximately 9.5%).

[0073] Referring to Table IV modified *B.t.k.* HD-1 genes were constructed that contained all of the above modifications (pMON5370) or various subsets of individual modifications. These genes were inserted ' into pMON893 for plant transformation and tobacco plants containing these genes were analyzed. The analysis of tobacco plants with the individual modifications was undertaken for several reasons. Expression of the wild type truncated gene in tobacco is very poor, resulting in infrequent identification of plants toxic to THW. Toxicity is defined by leaf feeding assays as at least 60% mortality of tobacco hornworm neonate larvae with a damage rating of 1 or less (scale is 0 to 4; 0 is equivalent to total protection, 4 total damage). The modified HD-1 gene (pMON5370) shows a large increase in expression (estimated to be approximately 100-fold; see Table VIII) in tobacco. Therefore, increases in expression of the wild-type gene due to indidvidual modifications would be apparently a large increase in the frequency of toxic tobacco plants and the presence of detectable *B.t.k.* protein. Results are shown in the following table:

Table IV

Relative effects of Regional Modifications within the *B.t.k.* Gene			
Construct	Position Modified	# of Plants	# of Toxic Plants
pMON5370	185, 240, 669, 930, 1110, 1380a+b, 1600	38	22
pMON10707	185, 240, 462, 669	48	19
pMON10706	930, 1110, 1380a+b, 1600	43	1
pMON10539	185	55	2
pMON10537	240	57	17
pMON10540	185, 240	88	23
pMON10705	462	47	1

[0074] The effects of each individual oligonucleotides' changes on expression did reveal some overall trends. Six

different constructs were generated which were designed to identify the key regions. The nine different oligonucleotides were divided in half by their position on the gene. Changes in the N-terminal half were incorporated into pMON10707 (185,240, 462,669). C-terminal half changes were incorporated into pMON10706 (930,1110,1380a+b,1600). The results of analysis of plants with these two constructs indicate that pMON10707 produces a substantial number of toxic plants (19 of 48). Protein from these plants is detectable by ELISA analysis. pMON10706 plants were rarely identified as insecticidal (1 of 43) and the levels of *B.t.k.* were barely detectable by immunological analysis. Investigation of the N-terminal changes in greater detail was done with 4 pMON constructs; 10539 (185 alone), 10537 (240 alone), 10540 (185 and 240) and 10705 (462 alone). The results indicate that the presence of the changes in 240 were required to generate a substantial number of toxic plants (pMON10540; 23 of 88, pMON10537; 17 of 57). The absence of the 240 changes resulted in a low frequency of toxic plants with low *B.t.k.* protein levels, identical to results with the wild type gene. These results indicate that the changes in 240 are responsible for a substantial increase in *B.t.k.* expression levels over an analogous wild-type construct in tobacco. Changes in additional regions (185,462,669) in conjunction with 240 may result in increases in *B.t.k.* expression (>2 fold). However, changes at the 240 region of the N-terminal portion of the gene do result in dramatic increases in expression.

[0075] Despite the importance of the alteration of the 240 region in expression of modified genes, increased expression can be achieved by alteration of other regions. Hybrid genes, part wild-type, part synthetic, were generated to determine the effects of synthetic gene segments on the levels of *B.t.k.* expression. A hybrid gene was generated with a synthetic N-terminal third (base pair 1 to 590 of Figure 2: to the XbaI site) with the C-terminal wild type *B.t.k.* HD-1 (pMON5378) Plants transformed with this vector were as toxic as plants transformed with the modified HD-1 gene (pMON5370). This is consistent with the alteration of the 240 region. However, pMON10538, a hybrid with a wild-type N-terminal third (wild type gene for the first 600 base pairs, to the second XbaI site) and a synthetic C-terminal last two-thirds (base pair 590 to 1845 of Figure 3 was used to transform tobacco and resulted in a dramatic increase in expression. The levels of expression do not appear to be as high as those seen with the synthetic gene, but are comparable to the modified gene levels. These results indicate that modification of the 240 segment is not essential to increased expression since pMON10538 has an intact 240 region. A fully synthetic gene is, in most cases, superior for expression levels of *B.t.k.* (See Example 2.)

Example 2 -- Fully Synthetic *B.t.k.* HD-1 Gene

[0076] A synthetic *B.t.k.* HD-1 gene was designed using the preferred plant codons listed in Table V below. Table V lists the codons and frequency of use in plant genes of dicotyledonous plants compared to the frequency of their use in the wild type *B.t.k.* HD-1 gene (amino acids 1-615) and the synthetic gene of this example. The total number of each amino acid in this segment of the gene is listed in the parenthesis under the amino acid designated.

Table V

Codon in Usage Synthetic *B.t.k.* HD-1 Gene				
Amino Acid	Codon	Percent Usage in Plants/Wt *B.t.k.*/Syn		
ARG (43)	CGA	7	11	2
	CGC	11	5	5
	CGG	5	2	0
	CGU	25	14	27
	AGA	29	55	41
	AGG	23	14	25
LE (49)	CUA	8	16	4
	CUC	20	0	20
	CUG	10	2	6
	CUU	28	22	24
	UUA	5	50	0
	UUG	30	10	45

Table V (continued)

Codon in Usage Synthetic *B.t.k.* HD-1 Gene				
Amino Acid	Codon	Percent Usage in Plants/Wt *B.t.k.*/Syn		
SER (64)	UCA	14	27	5
	UCC	26	9	28
	UCG	3	8	0
	UCU	21	19	31
	AGC	21	6	32
	AGU	15	31	5
THR (42)	ACA	21	31	14
	ACC	41	19	53
	ACG	7	14	0
	ACU	31	36	33
PRO (34)	CCA	45	35	53
	CCC	19	6	12
	CCG	9	21	3
	CCU	26	38	32
ALA (31)	GCA	23	38	26
	GCC	32	9	29
	GCG	3	3	0
	GCU	41	50	45
GLY (46)	GGA	32	52	45
	GGC	20	17	15
	GGG	11	15	6
	GGU	37	15	34
ILE (46)	AUA	12	39	2
	AUC	45	11	67
	AUU	43	50	30
VAL (38)	GUA	9	45	3
	GUC	20	5	16
	GUG	28	11	37
	GUU	43	39	45
LYS (3)	AAA	36	100	33
	AAG	64	0	67
ASN (44)	AAC	72	27	80
	AAU	28	73	20

Table V (continued)

Codon in Usage Synthetic *B.t.k.* HD-1 Gene				
Amino Acid	Codon	Percent Usage in Plants/Wt *B.t.k.*/Syn		
GLN (31)	CAA	64	77	61
	CAG	36	23	39
HIS (10)	CAC	65	0	80
	CAU	35	100	20
GLU (30)	GAA	48	87	50
	GAG	52	13	50
ASP (23)	GAC	48	17	65
	GAU	52	83	35
TYR (25)	UAC	68	20	72
	UAU	32	80	28
CYS (2)	UGC	78	50	100
	UGU	22	50	0
PHE (36)	UUC	56	17	83
	UUU	44	83	17
MET (9)	AUG	100	100	100
TRP (9)	UGG	100	100	100

[0077] The resulting synthetic gene lacks ATTTA sequences, contains only one potential polyadenylation site and has a G+C content of 48.5%. Figure 3 is a comparison of the wild-type HD-1 sequence to the synthetic gene sequence for amino acids 1-615. There is approximately 77% DNA homology between the synthetic gene and the wild-type gene and 356 of the 615 codons have been changed (approximately 60%).

Example 3 -- Synthetic *B.t.k.* HD-73 Gene

[0078] The crystal protein toxin from *B.t.k.* HD-73 exhibits a higher unit activity against some important agricultural pests. The toxin protein of HD-1 and HD-73 exhibit substantial homology (~90%) in the N-terminal 450 amino acids, but differ substantially in the amino acid region 451-615. Fusion proteins comprising amino acids 1-450 of HD-1 and 451-615 of HD-73 exhibit the insecticidal properties of the wild-type HD-73. The strategy employed was to use the 5'-two thirds of the synthetic HD-1 gene (first 1350 bases, up to the SacI site) and to dramatically modify the final 590 bases (through amino acid 645) of the HD-73 in a manner consistent with the algorithm used to design the synthetic HD-1 gene. Table VI below lists the oligonucleotides used to modify the HD-73 gene in the order used in the gene from 5' to 3' end. Nine oligonucleotides were used in a 590 base pair region, each nucleotide ranging in size from 33 to 60 bases. The only regions left unchanged were areas where there were no long consecutive strings of A or T bases (longer than six). All polyadenylation sites and ATTTA sites were eliminated.

Table VI

Mutagenesis Primers for *B.t.k.* HD-73

Primer	Length (bp)	Sequence
73K1363	51	AATACTATCG GATGCGATGA TGTTGTTGAA CTCAGCACTA CGGTGTATCC A
73K1437	33	TCCTGAAATG ACAGAACCGT TGAAGAGAAA GTT
73K1471	48	ATTTCCACTG CTGTTGAGTC TAACGAGGTC TCCACCAGTG AATCCTGG
73K1561	60	GTGAATAGGG GTCACAGAAG CATACCTCAC ACGAACTCTA TATCTGGTAG ATGTTGGATGG
73K1642	33	TGTAGCTGGA ACTGTATTGG AGAAGATGGA TGA
73K1675	48	TTCAAAGTAA CCGAAATCGC TGGATTGGAG ATTATCCAAG GAGGTAGC
73K1741	39	ACTAAAGTTT CTAACACCCA CGATGTTACC GAGTGAAGA

Table VI - continued

Mutagenesis Primers for *B.t.k.* HD-73

Primer	Length (bp)	Sequence
73K1797	36	AACTGGAATG AACTCGAATC TGTCGATAAT CACTCC
73KTERM	54	GGACACTAGA TCTTAGTGAT AATCGGTCAC ATTTGTCTTG AGTCCAAGCT GGTT

[0079] The resulting gene has two potential polyadenylation sites (compared to 18 in the WT) and no ATTTA sequence (12 in the WT). The G+C content has increased from 37% to 48%. A total of 59 individual base pair changes were made using the primers in Table VI. Overall, there is 90% DNA homology between the region of the HD-73 gene modified by site directed mutagenesis and the wild-type sequence of the analogous region of HD-73. The synthetic HD-73 is a hybrid of the first 1360 bases from the synthetic HD-1 and the next 590 bases or so modified HD-73 sequence. Figure 4 is a comparison of the above-described synthetic *B.t.k.* HD-73 and the wild-type *B.t.k.* HD-73 encoding amino acids 1-645. In the modified region of the HD-73 gene 44 of the 170 codons (25%) were changed as a result of the site-directed mutagenesis changes resulting from the oligonucleotides found in Table VI. Overall, approximately 50% of the codons in the synthetic *B.t.k.* HD-73 differ from the analogous segment of the wild-type and HD-73 gene.

[0080] A one base pair deletion in the synthetic HD-73 gene was detected in the course of sequencing the 3' end at base pair 1890. This results in a frame-shift mutation at amino acid 625 with a premature stop codon at amino acid 640 (pMON5379). Table VII below compares the codon usage of the wild-type gene of *B.t.k.* HD-73 versus the synthetic gene of this example for amino acids 451-645 and codon usage of naturally occurring genes of dicotyledonous plants. The total number of each amino acid encoded in this segment of the gene is found in the parentheses under the amino acid designation.

Table VII

Codon Usage in Synthetic B.t.k. HD-73 Gene				
Amino Acid	Codon	Percent Usage in Plants/Wt HD-73/Syn		
ARG (10)	CGA	7	10	0
	CGC	11	0	8
	CGG	5	10	0
	CGU	25	20	23
	AGA	29	60	62
	AGG	23	0	8

Table VII (continued)

Codon Usage in Synthetic B.t.k. HD-73 Gene				
Amino Acid	Codon	Percent Usage in Plants/Wt HD-73/Syn		
LEU (12)	CUA	8	25	8
	CUC	20	17	58
	CUG	10	17	8
	CUU	28	8	0
	UUA	5	33	8
	UUG	30	0	17
SER (21)	UCA	14	24	18
	UCC	26	10	27
	UCG	3	10	0
	UCU	21	24	18
	AGC	21	0	14
	AGU	15	33	23
THR (15)	ACA	21	47	38
	ACC	41	13	31
	ACG	7	13	0
	ACU	31	27	31
PRO (7)	CCA	45	71	71
	CCC	19	0	0
	CCG	9	14	0
	CCU	26	14	29
ALA (14)	GCA	23	29	31
	GCC	32	7	8
	GCG	3	21	15
	GCU	41	43	46
GLY (15)	GGA	32	33	43
	GGC	20	0	0
	GGG	11	27	14
	GGU	37	40	43
ILE (15)	AUA	12	33	7
	AUC	45	7	40
	AUU	43	60	53

Table VII (continued)

Codon Usage in Synthetic B.t.k. HD-73 Gene				
Amino Acid	Codon	Percent Usage in Plants/Wt HD-73/Syn		
VAL (15)	GUA	9	40	7
	GUC	20	0	7
	GUG	28	20	36
	GUU	43	40	50
LYS (3)	AAA	36	67	100
	AAG	64	33	0
ASN (20)	AAC	72	20	53
	AAU	28	80	47
GLN (5)	CAA	64	60	67
	CAG	36	40	33
HIS (3)	CAC	65	67	100
	CAU	35	33	0
GLU (7)	GAA	48	86	57
	GAG	52	14	43
ASP (5)	GAC	48	40	50
	GAU	52	60	50
TYR (5)	UAC	68	0	20
	UAU	32	100	80
CYS (0)	UGC	78	0	0
	UGU	22	0	0
PHE (13)	UUC	56	8	67
	UUU	44	92	33
MET (2)	AUG	100	100	100
TRP (2)	UGG	100	100	100

[0081] Another truncated synthetic HD-73 gene was constructed. The sequence of this synthetic HD-73 gene is identical to that of the above synthetic HD-73 gene in the region in which they overlap (amino acids 29-615), and it also encodes Met-Ala at the N-terminus. Figure 8 shows a comparison of this truncated synthetic HD-73 gene with the N-terminal Met-Ala versus the wild-type HD-73 gene.

[0082] While the previous examples have been directed at the preparation of synthetic and modified genes encoding truncated *B.t.k.* proteins, synthetic or modified genes can also be prepared which encode full length toxin proteins.

[0083] One full length *B.t.k.* gene consists of the synthetic HD-73 sequence of Figure 4 from nucleotide 1-1845 plus wild-type HD-73 sequence encoding amino acids 616 to the C-terminus of the native protein. Figure 9 shows a com-

parison of this synthetic/wild-type full length HD-73 gene versus the wild-type full length HD-73 gene.

[0084] Another full length *B.t.k.* gene consists of the synthetic HD-73 sequence of Figure 4 from nucleotide 1-1845 plus a modified HD-73 sequence ending amino acids 616 to the C-terminus of the native protein. The C-terminal portion has been modified by site-directed mutagenesis to remove putative polyadenylation signals and ATTTA sequences according to the algorithm of Figure 1. Figure 10 shows a comparison of this synthetic/modified full length HD-73 gene versus the wild-type full length HD-73 gene.

[0085] Another full length *B.t.k.* gene consists of a fully synthetic HD-73 sequence which incorporates the synthetic HD-73 sequence of Figure 4 from nucleotide 1-1845 plus a synthetic sequence encoding amino acids 616 to the C-terminus of the native protein. The C-terminal synthetic portion has been designed to eliminate putative polyadenylation signals and ATTTA sequences and to include plant preferred codons. Figure 11 shows a comparison of this fully synthetic full length HD-73 gene versus the wild-type full length HD-73 gene.

[0086] Alternatively, another full length *B.t.k.* gene consists of a fully synthetic sequence comprising base pairs 1-1830 of *B.t.k.* HD-1 (Figure 3) and base pairs 1834-3534 of *B.t.k.* HD-73 (Figure 11).

<u>Example 4 – Expression of Modified and Synthetic *B.t.k.* HD-1 and Synthetic HD-73</u>

[0087] A number of plant transformation vectors for the expression of *B.t.k.* genes were constructed by incorporating the structural coding sequences of the previously described genes into plant transformation cassette vector pMON893. The respective intermediate transformation vector is inserted into a suitable disarmed *Agrobacterium* vector such as *A. tumefaciens* ACO, supra. Tissue explants are cocultured with the disarmed *Agrobacterium* vector and plants regenerated under selection for kanamycin resistance using known protocols: tobacco (Horsch et al., 1985); tomato (McCormick et al., 1986) and cotton (Trolinder et al., 1987).

a) Tobacco.

[0088] The level of *B.t.k.* HD-1 protein in transgenic tobacco plants containing pMON9921 (wild type truncated), pMON5370 (modified HD-1, Example 1, Figure 2) and pMON5377 (synthetic HD-1, Example 2, Figure 3) were analyzed by Western analysis. Leaf tissue was frozen in liquid nitrogen, ground to a fine powder and then ground in a 1:2 (wt: volume) of SDS-PAGE sample buffer. Samples were frozen on dry ice, then incubated for 10 minutes in a boiling water bath and microfuged for 10 minutes. The protein concentration of the supernatant was determined by the method of Bradford (Anal. Biochem. 72:248-254). Fifty ug of protein was run per lane on 9% SDS-PAGE gels, the protein transferred to nitrocellulose and the *B.t.k.* HD-1 protein visualized using antibodies produced against *B.t.k.* HD-1 protein as the primary antibody and alkaline phosphatase conjugated second antibody as described by the manufacturer (Promega, Madison, WI). Purified HD-1 tryptic fragment was used as the control. Whereas the *B.t.k.* protein from tobacco plants containing pMON9921 was below the level of detection, the *B.t.k.* protein from plants containing the modified (pMON5370) and synthetic (pMON5377) genes was easily detected. The *B.t.k.* protein from plants containing pMON9921 remained undetectable, even with 10 fold longer incubation times. The relative levels of *B.t.k.* HD-1 protein in these plants is estimated in Table VIII. Because the protein from plants containing pMON9921 was not observed, the level of protein in these plants was estimated from the relative mRNA levels (see below). Plants containing the modified gene (pMON5370) expressed approximately 100 fold more *B.t.k.* protein than plants containing the wild-type gene (pMON9921). Plants containing the fully synthetic *B.t.k.* HD-1 gene (pMON5377) expressed approximately five fold more protein than plants containing the modified gene. The modified gene contributes the majority of the increase in *B.t.k.* expression observed. The plants used to generate the above data are the best representatives from each construct based either on a tobacco hornworm bioassay or on data derived from previous Western analysis.

Table VIII

Expression of *B.t.k.* HD-1 Protein in Transgenic Tobacco			
Gene Description	Vector	*B.t.k.* Protein* Concentration	Fold Increase in *B.t.k.* Expression
Wild type	pMON9921	10	1
Modified	pMON5370	1000	100
Synthetic	pMON5377	5000	500

* *B.t.k.* protein concentrations are expressed in ng/mg of total soluble protein. The level of *B.t.k.* protein for plants containing the wild type gene are estimated from mRNA levels.

[0089] Plants containing these genes were tested for bioactivity to determine whether the increased quantities of

protein observed by Western analysis result in a corresponding increase in bioactivity. Leaves from the same plants used for the Western data in Table 1 were tested for bioactivity against two insects. A detached leaf bioassay was first done using tobacco hornworm, an extremely sensitive lepidopteran insect. Leaves from all three transgenic tobacco plants were totally protected and 100% mortality of tobacco hornworm observed (see Table IX below). A much less sensitive insect, beet armyworm, was then used in another detached leaf bioassay. Beet armyworm is approximately 500 fold less sensitive to *B.t.k.* HD-1 protein than tobacco hornworm. The difference in sensitivity of these two insects was determined using purified HD-1 protein in a diet incorporation assay (see below). Plants containing the wild-type gene (pMON9921) showed only minimal protection against beet armyworm, whereas plants containing the modified gene showed almost complete protection and plants containing the fully synthetic gene were totally protected against beet armyworm damage. The results of these bioassays confirm the levels of *B.t.k.* HD-1 expression observed in the Western analysis and demonstrates that the increased levels of *B.t.k.* HD-1 protein correlates with increased insecticidal activity.

Table IX

Protection of Tobacco Plants from Tobacco Hornworm and Beet Armyworm			
Gene Description	Vector	Tobacco Hornworm Damage*	Beet Armyworm Damage*
None	None	NL	NL
Wild type	pMON9921	0	3
Modified	pMON5370	0	1
Synthetic	pMON5377	0	0

* Extent of insect damage was rated: 0, no damage; 1, slight; 2, moderate; 3, severe; or NL, no leaf left.

[0090] The bioactivity of the *B.t.k.* HD-1 protein produced by these transgenic plants was further investigated to more accurately quantitate the relative activities. Leaf tissue from tobacco plants containing the wild-type, modified and synthetic genes were ground in 100 mM sodium carbonate buffer, pH 10 at a 1:2 (wt:vol) ratio. Particulate material was removed by centrifugation. The supernatant was incorporated into a synthetic diet similar to that described by Marrone et al. (1985). The diet medium was prepared the day of the test with the plant extract solutions incorporated in place of the 20% water component. One ml of the diet was aliquoted into 96 well plates.

[0091] After the diet dried, one neonate tobacco budworm larva was added to each well. Sixteen insects were tested with each plant sample. The plants were incubated at 27°C. After seven days, the larvae from each treatment were combined and weighed on an analytical balance. The average weight per insect was calculated and compared to a standard curve relating *B.t.k.* protein concentrations to average larval weight. Insect weight was inversely proportional (in a logarithmic manner) to the relative increase in *B.t.k.* protein concentration. The amount of *B.t.k.* HD-1 protein, based on the extent of larval growth inhibition was determined for two different plants containing each of the three genes. The specific activity (ng of *B.t.k.* HD-1 per mg of plant protein) was determined for each plant. Plants containing the modified HD-1 gene (pMON5370) averaged approximately 1400 ng (1200 and 1600 ng) of *B.t.k.* HD-1 per mg of plant extract protein. This value compares closely with the 1000 ng of *B.t.k.* HD-1 protein per mg of plant extract protein as determined by Western analysis (Table I). *B.t.k.* HD-1 concentrations for the plants containing the synthetic HD-1 gene averaged approximately 8200 ng (7200 and 9200 ng) of *B.t.k.* HD-1 protein per mg of plant extract protein. This number compares well to the 5000 ng of HD-1 protein per mg of plant extract protein estimated by Western analysis. Likewise, plants containing the synthetic gene showed approximately a six-fold higher specific activity than the corresponding plants containing the modified gene for these bioassays. In the Western analysis the ratio was approximately 10 fold, again both are in good agreement. The level of *B.t.k.* protein in plants containing the wild-type HD-1 gene (pMON9921) was too low to give a significant decrease in larval weight and hence was below a level that could be quantitated in this assay. In conclusion, the levels of *B.t.k.* HD-1 protein determined by both the bioassays and the Western analysis for these plants containing the modified and synthetic genes agree, which demonstrates that the *B. t.k.* HD-1 protein produced by these plants is biologically active.

[0092] The levels of mRNA were determined in the plants containing the wild-type *B.t.k.* HD-1 gene (pMON9921) and the modified gene (pMON5370) to establish whether the increased levels of protein production result from increased transcription or translation. mRNA from plants containing the synthetic gene could not be analyzed directly with the same DNA probe as used for the wild-type and modified genes because of the numerous changes made in the coding sequence. mRNA was isolated and hybridized with a single-stranded DNA probe homologous to approximately the 5' 90 bp of the wild-type or modified gene coding sequences. The hybrids were digested with S1 nuclease and the protected probe fragments analyzed by gel electrophoresis. Because the procedure used a large excess of probe and long hybridization time, the amount of protected probe is proportional to the amount of *B.t.k.* mRNA present in the sample. Two plants expressing the modified gene (pMON5370) were found to produce up to ten-fold more RNA

than a plant expressing the wild-type gene (pMON9921).

[0093] The increased mRNA level from the modified gene is consistent with the result expected from the modifications introduced into this gene. However, this 10 fold increase in mRNA with the modified gene compared to the wild-type gene is in contrast to the 100 'fold increase in *B.t.k.* protein from these genes in tobacco plants. If the two mRNAs were equally well translated then a 10 fold increase in stable mRNA would be expected to yield a 10 fold increase in protein. The higher increase in protein indicates that the modified gene mRNA is translated at about a 10 fold higher efficiency than wild-type. Thus, about half of the total effect on gene expression can be explained by changes in mRNA levels and about half to changes in translational efficiency. This increase in translational efficiency is striking in that only about 9.5% of the codons have been changed in the modified gene; that is, this effect is clearly not due to wholesale codon usage changes. The increased translational efficiency could be due to changes in mRNA secondary structure that affect translation or to the removal of specific translational blockades due to specific codons that were changed.

[0094] The increased expression seen with the synthetic HD-1 gene was also seen with a synthetic HD-73 gene in tobacco. *B.t.k.* HD-73 was undetected in extracts of tobacco plants containing the wild-type truncated HD-73 gene (pMON5367), whereas *B.t.k.* HD-73 protein was easily detected in extracts from tobacco plants containing the synthetic HD-73 gene of Figure 4 (pMON5383). Approximately 1000 ng of *B.t.k.* HD-73 protein was detected per mg of total soluble plant protein.

[0095] As described in Example 3 above, the *B.t.k.* HD-73 protein encoded in pMON5383 contains a small C-terminal extension of amino acids not encoded in the wild-type HD-73 protein. These extra amino acids had no effect on insect toxicity or on increased plant expression. A second synthetic HD-73 gene was constructed as described in Example 3 (Figure 8) and used to transform tobacco (pMON5390). Analysis of plants containing pMON5390 showed that this gene was expressed at levels comparable to that of pMON5383 and that these plants had similar insecticidal efficacy.

[0096] In tobacco plants the synthetic HD-1 gene was expressed at approximately a 5-fold higher level than the synthetic HD-73 gene. However, this synthetic HD-73 gene still was expressed at least 100-fold better than the wild-type HD-73 gene. The HD-73 protein is approximately 5-fold more toxic to many insect pests than the HD-1 protein, so both synthetic HD-1 and HD-73 genes provide approximately comparable insecticidal efficacy in tobacco.

[0097] The full length *B.t.k.* HD-73 genes described in Example 3 were also incorporated into the plant transformation vector pMON893 so that they were expressed from the En 35S promoter. The synthetic/wild-type full length HD-73 gene of Figure 9 was incorporated into pMON893 to create pMON10505. The synthetic/modified full length HD-73 gene of Figure 10 was incorporated into pMON893 to create pMON10526. The fully synthetic HD-73 gene of Figure 11 was incorporated into pMON893 to create pMON10518. These vectors were used to obtain transformed tobacco plants, and the plants were analyzed for insecticidal efficacy and for *B.t.k.* HD-73 protein levels by Western blot or ELISA immunoassay.

[0098] Tobacco plants containing all three of these full length *B.t.k.* genes produced detectable *B.t.k.* protein and showed 100% mortality of tobacco hornworm. This result is surprising in light of previous reported attempts to express the full length B.t.k. genes in transgenic plants. Vaeck et al. (1987) reported that a full length *B.t.k. berliner* gene similar to our HD-1 gene could not be detectably expressed in tobacco. Barton et al. (1987) reported a similar result for another full length gene from *B.t.k.* HD-1 (the so called 4.5 kb gene), and further indicated that tobacco callus containing this gene became necrotic, indicating that the full length gene product was toxic to plant cells. Fischhoff et al. (1987) reported that the full length *B.t.k.* HD-1 gene in tomato was poorly expressed compared to a truncated gene, and no plants that were fully toxic to tobacco hornworm could be recovered. All three of the above reports indicated much higher expression levels and recovery of toxic plants if the respective *B.t.k.* genes were truncated. Adang et al. reported that the full length HD-73 gene yielded a few tobacco plants with some biological activity (none were highly toxic) against hornworm and barely detectable *B.t.k.* protein. It was also noted by them that the major *B.t.k.* mRNA in these plants was a truncated 1.7 kb species that would not encode a functional toxin. This indicated improper expression of the gene in tobacco. In contrast to all of these reports, the three full length *B.t.k.* HD-73 genes described above all lead to relatively high levels of protein and high levels of insect toxicity.

[0099] *B.t.k.* protein and mRNA levels in tobacco plants are shown in Table X for these three vectors. As can be seen from the table, the synthetic/wild-type gene (pMON10506) produces *B.t.k.* protein as about 0.01% of total soluble protein; the synthetic/modified gene produces *B.t.k.* as about 0.02% of total soluble protein; and the fully synthetic gene produces *B.t.k.* as about 0.2% of total soluble protein. *B.t.k.* mRNA was analyzed in these plants by Northern blot analysis using the common 5' synthetic half of the genes as a probe. As shown in Table X, the increased protein levels can largely be attributed to increased mRNA levels. Compared to the truncated modified and synthetic genes, this could indicate that the major contributors to increased translational efficiency are in the 5' half of the gene while the 3' half of the gene contains mostly determinants of mRNA stability. The increased protein levels also indicate that increasing the amount of the full length gene that is synthetic or modified increases *B.t.k.* protein levels. Compared to the truncated synthetic *B.t.k.* HD-73 genes (pMON5383 or pMON5390), the fully synthetic gene (pMON10518) produces as much or slightly more *B.t.k.* protein demonstrating that the full length genes are capable of being expressed at high levels in plants. These tobacco plants with high levels of full length HD-73 protein show no evidence of abnor-

mality and are fully fertile. The *B.t.k.* protein levels in these plants also produce the expected levels of insect toxicity based on feeding studies with beet armyworm or diet incorporation assays of plant extracts with tobacco budworm. The *B.t.k.* protein detected by Western blot analysis in these tobacco plants often contains a varying amount of protein of about 80 kDa which is apparently a proteolytic fragment of the full length protein. The C-terminal half of the full length protein is known to be proteolytically sensitive, and similar proteolytic fragments are seen from the full length gene in *E. coli* and *B.t.* itself. These fragments are fully insecticidal. The Northern analysis indicated that essentially all of the mRNA from these full length genes was of the expected full length size. There is no evidence of truncated mRNAs that could give rise to the 80 kDa protein fragment. In addition, it is possible that the fragment is not present in intact plant cells and is merely due to proteolysis during extraction for immunoassay.

Table X

Full Length *B.t.k.* HD-73 Protein and mRNA Levels in Transgenic Tobacco Plants			
Gene description	Vector	B.t.k. protein concentration	Relative B.t.k. mRNA level
Synthetic/wild type	pMON10506	>100	0.5
Synthetic/modified	pMON10526	400	1
Fully synthetic	pMON10518	>2000	40

[0100] Thus, there is no serious impediment to producing high levels of *B.t.k.* HD-73 protein in plants from synthetic genes, and this is expected to be true of other full length lepidopteran active genes such as *B.t.k.* HD-1 or *B.t. entomocidus.* The fully synthetic B.t.k. HD-1 gene of Example 3 has been assembled in plant transformation vectors such as pMON893.

[0101] The fully synthetic gene in pMON10518 was also utilized in another plant vector and analyzed in tobacco plants. Although the CaMV35S promoter is generally a high level constitutive promoter in most plant tissues, the expression level of genes driven the CaMV35S promoter is low in floral tissue relative to the levels seen in leaf tissue. Because the economically important targets damaged by some insects are the floral parts or derived from floral parts (e.g., cotton squares and bolls, tobacco buds, tomato buds and fruit), it may be advantageous to increase the expression of *B.t.* protein in these tissues over that obtained with the CaMV35S promoter.

[0102] The 35S promoter of Figwort Mosaic Virus (FMV) is analogous to the CaMV35S promoter. This promoter has been isolated and engineered into a plant transformation vector analogous to pMON893. Relative to the CaMV promoter, the FMV 35S promoter is highly expressed in the floral tissue, while still providing similar high levels of gene expression in other tissues such as leaf. A plant transformation vector, pMON10517, was constructed in which the full length synthetic *B.t.k.* HD-73 gene of Figure 11 was driven by the FMV 35S promoter. This vector is identical to pMON10518 of Example 3 except that the FMV promoter is substituted for the CaMV promoter. Tobacco plants transformed with pMON10517 and pMON10518 were obtained and compared for expression of the *B.t.k.* protein by Western blot or ELISA immunoassay in leaf and floral tissue. This analysis showed that pMON10517 containing the FMV promoter expressed the full length HD-73 protein at higher levels in floral tissue than pMON10518 containing the CaMV promoter. Expression of the full length *B.t.k.* HD-73 protein from pMON10517 in leaf tissue is comparable to that seen with the most highly expressing plants containing pMON10518. However, when floral tissue was analyzed, tobacco plants containing pMON10518 that had high levels of *B.t.k.* protein in leaf tissue did not have detectable *B.t.k.* protein in the flowers. On the other hand, flowers of tobacco plants containing pMON10517 had levels of *B.t.k.* protein nearly as high as the levels in leaves at approximately 0.05% of total soluble protein. This analysis showed that the FMV promoter could be used to produce relatively high levels of *B.t.k.* protein in floral tissue compared to the CaMV promoter.

b) Tomato.

[0103] The wild-type, modified and synthetic *B.t.k.* HD-1 genes tested in tobacco were introduced into other plants to demonstrate the broad utility of this invention. Transgenic tomatoes were produced which contain these three genes. Data show that the increased expression observed with the modified and synthetic gene in tobacco also extends to tomato. Whereas the *B.t.k.* HD-1 protein is only barely detectable in plants containing the wild type HD-1 gene (pMON9921), *B.t.k.* HD-1 was readily detected and the levels determined for plants containing the modified (pMON5370) or synthetic (pMON5377) genes. Expression levels for the plants containing the wild-type, modified and synthetic HD-1 genes were approximately 10, 100 and 500 ng per mg of total plant extract see Table XI below). The increase in *B.t.k.* HD-1 protein for the modified gene accounted for the majority of increase observed; 10 fold higher than the plants containing the wild-type gene, compared to only an additional five-fold increase for plants containing the synthetic gene. Again the site-directed changes made in the modified gene are the major contributors to the increased expression of *B.t.k.* HD-1.

Table XI

B.t.k. HD-1 Expression in Transgenic Tomato Plants			
Gene Description	Vector	B.t.k. Protein* Concentration	Fold Increase in B.t.k. Expression
Wild type	pMON9921	10	1
Modified	pMON5370	100	10
Synthetic	pMON5377	500	50

* *B.t.k.* HD-1 protein concentrations are expressed in ng/mg of total soluble plant protein. Data for plants containing the wild-type gene are estimates from mRNA levels and protein levels determined by ELISA.

[0104] These differences in *B.t.k.* HD-1 expression were confirmed with bioassays against tobacco hornworm and beet armyworm. Leaves from tomato plants containing each of these genes controlled tobacco hornworm damage and produced 100% mortality. With beet armyworm, leaves from plants containing the wild-type HD-1 gene (pMON9921) showed significant damage, leaves from plants containing the modified gene (pMON5370) showed less damage and leaves from plants containing the synthetic gene (pMON5377) were completely protected (see Table XII below).

Table XII

Protection of Tomato Plants from Tobacco Hornworm and Beet Armyworm			
Gene Description	Vector	Tobacco Hornworm Damage*	Beet Armyworm Damage*
None	None	NL	NL
Wild type	pMON9921	0	3
Modified	pMON5370	0	1
Synthetic	pMON5377	0	0

* Damage was rated as shown in Table IX.

[0105] The generality of the synthetic gene approach was extended in tomato with a synthetic *B.t.k.* HD-73 gene.

[0106] In tomato, extracts from plants containing the wild-type truncated HD-73 gene (pMON5367) showed no detectable HD-73 protein. Extracts from plants containing the synthetic HD-73 gene (pMON5383) showed high levels of *B.t.k.* HD-73 protein, approximately 2000 ng per mg of plant extract protein. These data clearly demonstrate that the changes made in the synthetic HD-73 gene lead to dramatic increases in the expression of the HD-73 protein in tomato as well as in tobacco

[0107] In contrast to tobacco, the synthetic HD-73 gene in tomato is expressed at approximately 4-fold to 5-fold higher levels than the synthetic HD-1 gene. Because the HD-73 protein is about 5-fold more active than the HD-1 protein against many insect pests including Heliothis species, the increased expression of synthetic HD-73 compared to synthetic HD-1 corresponds to about a 25-fold increased insecticidal efficacy in tomato.

[0108] In order to determine the mechanisms involved in the increased expression of modified and synthetic *B.t.k.* HD-1 genes in tomato, S1 nuclease analysis of mRNA levels from transformed tomato plants was performed. As indicated above, a similar analysis had been performed with tobacco plants, and this analysis showed that the modified gene produced up to 10-fold more mRNA than the wild-type gene. The analysis in tomato utilized a different DNA probe that allowed the analysis of wild-type (pMON9921), modified (pMON5370) and synthetic (pMON5377) HD-1 genes with the same probe. This probe was derived from the 5' untranslated region of the CaMV35S promoter in pMON893 that was common to all three of these vectors (pMON9921, pMON5370 and pMON5377). This S1 analysis indicated that *B.t.k.* mRNA levels from the modified gene were 3 to 5 fold higher than for the wild-type gene, and that mRNA levels for the synthetic gene were about 2 to 3 fold higher than for the modified gene. Three independent transformants were analyzed for each gene. Compared to the fold increases in *B.t.k.* HD-1 protein from these genes in tomato shown in Table XI, these mRNA increases can explain about half of the total protein increase as was seen in tobacco for the wild-type and modified genes. For tomato the total mRNA increase from wild-type to synthetic is about 6 to 15 fold compared to a protein increase of about 50 fold. This result is similar to that seen for tobacco in comparing the wild-type and modified genes, and it extends to the synthetic gene as well. That is, about half of the total fold increase in *B.t.k.* protein from wild-type to modified genes can be explained by mRNA increases and about half to enhanced translational efficiency. The same is also true in comparing the modified gene to the synthetic gene. Although there is an additional increase in RNA levels, this mRNA increase can explain only about half of the total protein increase.

[0109] The full length *B.t.k.* genes described above were also used to transform tomato plants and these plants were

analyzed for *B.t.k.* protein and insecticidal efficacy. The results of this analysis are shown in Table XIII. Plants containing the synthetic/wild-type gene (pMON10506) produce the *B.t.k.* HD-73 protein at levels of about 0:01% of their total soluble protein. Plants containing the synthetic/modified gene (pMON10526) produce about 0.04% *B.t.k.* protein, and plants containing the fully synthetic gene (pMON10518) produce about 0.2% *B.t.k.* protein. These results are very similar to the tobacco plant results for the same genes. mRNA levels estimated by Northern blot analysis in tomato also increase in parallel with the protein level increase. As for tobacco with these three genes, most of the protein increase can be attributed to increased mRNA with a small component of translational efficiency increase indicated for the fully synthetic gene. The highest levels of full length *B.t.k.* protein (from pMON10518) are comparable to or just slightly lower than the highest levels observed for the truncated HD-73 genes (pMON5383 and pMON5390). Tomato plants expressing these full length genes have the insecticidal activity expected for the observed protein levels as determined by feeding assays with beet armyworm or by diet incorporation of plant extracts with tobacco hornworm.

Table XIII

Full Length *B.t.k.* HD-73 Protein and mRNA Levels in Transgenic Tomato Plants			
Gene description	Vector	*B.t.k.* protein concentration	Relative *B.t.k.* mRNA level
Synthetic/wild type	pMON10506	100	1
Synthetic/modified	pMON10526	400	2-4
Fully synthetic	pMON10518	2000	10

c) Cotton.

[0110] The generality of the increased expression of *B.t.k.* HD-1 and *B.t.k.* HD-73 by use of the modified and synthetic genes was extended to cotton. Transgenic calli were produced which contain the wild type (pMON9921) and the synthetic HD-1 (pMON5377) genes. Here again the *B.t.k.* HD-1 protein produced from calli containing the wild-type gene was not detected, whereas calli containing the synthetic HD-1 gene expressed the HD-1 protein at easily detectable levels. The HD-1 protein was produced at approximately 1000 ng/mg of plant calli extract protein. Again, to ensure that the protein produced by the transgenic cotton calli was biologically active and that the increased expression observed with the synthetic gene translated to increased biological activity, extracts of cotton calli were made in similar manner as described for tobacco plants, except that the calli was first dried between Whatman filter paper to remove as much of the water as possible. The dried calli were then ground in liquid nitrogen and ground in 100 mM sodium carbonate buffer, pH 10. Approximately 0.5 ml aliquotes of this material was applied to tomato leaves with a paint brush. After the leaf dried, five tobacco hornworm larvae were applied to each of two leaf samples. Leaves painted with extract from control calli were completely destroyed. Leaves painted with extract from calli containing the wild-type HD-1 gene (pMON9921) showed severe damage. Leaves painted with extract from calli containing the synthetic HD-1 gene (pMON5377) showed no damage (see Table XIV below).

Table XIV

Protection against Tobacco Hornworm by Tomato Leaves Painted with Extracts Prepared from Cotton Calli Containing a Control, the Wild-Type *B.t.k.* HD-1 Gene. Synthetic HD-1 Gene or Synthetic HD-73 Gene		
Gene Description	Vector	Tobacco Hornworm Damage*
Control	Control	NL
Wild type HD-1	pMON9921	3
Synthetic HD-1	pMON5377	0
Synthetic HD-73	pMON5383	0

* Damage was rated as shown in Table IX.

[0111] Cotton calli were also produced containing another synthetic gene, a gene encoding *B.t.k.* HD-73. The preparation of this gene is described in Example 3. Calli containing the synthetic HD-73 gene produced the corresponding HD-73 protein at even higher levels than the calli which contained the synthetic HD-1 gene. Extracts made from calli containing the HD-73 synthetic gene (pMON5383) showed complete control of tobacco hornworm when painted onto tomato leaves as described above for extracts containing the HD-1 protein. (See Table XIV).

[0112] Transgenic cotton plants containing the synthetic *B.t.k.* HD-1 gene (pMON5377) or the synthetic *B.t.k.* HD-73 gene (pMON5383) have also been examined. These plants produce the HD-1 or HD-73 proteins at levels comparable to that seen in cotton callus with the same genes and comparable to tomato and tobacco plants with these genes.

For either synthetic truncated HD-1 or HD-73 genes, cotton plants expressing *B.t.k.* protein at 1000 to 2000 ng/mg total protein (0.1% to 0.2%) were recovered at a high frequency. Insect feeding assays were performed with leaves from cotton plants expressing the synthetic HD-1 or HD-73 genes. These leaves showed no damage (rating of 0) when challenged with larvae of cabbage looper (Trichoplusia ni), and only slight damage when challenged with larvae of beet armyworm (Spodoptera exigua). Damage ratings are as defined in Table IX above. This demonstrated that cotton plants as well as calli expressed the synthetic HD-1 or HD-73 genes at high levels and that those plants were protected from damage by Lepidopteran insect larvae.

[0113] Transgenic cotton plants containing either the synthetic truncated HD-1 gene (pMON5377) or the synthetic truncated HD-73 gene (pMON5383) were also assessed for protection against cotton bollworm at the whole plant level in the greenhouse. This is a more realistic test of the ability of these plants to produce an agriculturally acceptable level of control. The cotton bollworm (Heliothis zea) is a major pest of cotton that produces economic damage by destroying terminals, squares and bolls, and protection of these fruiting bodies as well as the leaf tissue will be important for effective insect control and adequate crop protection. To test the protection afforded to whole plants, R1 progeny of cotton plants expressing high levels of either *B.t.k.* HD-1 (pMON5377) or *B.t.k.* HD-73 (pMON5383) were assayed by applying 10-15 eggs of cotton bollworm per boll or square to the 20 uppermost squares or bolls on each plant. At least 12 plants were analyzed per treatment. 'The hatch rate of the eggs was approximately 70%. This corresponds to very high insect pressure compared to numbers of larvae per plant seen under typical field conditions. Under these conditions 100% of the bolls on control cotton plants were destroyed by insect damage. For the transgenics, significant boll protection was observed. Plants containing pMON5377 (HD-1) had 70-75% of the bolls survive the intense pressure of this assay. Plants containing pMON5383 (HD-73) had 80% to 90% boll protection. This is likely to be a consequence of the higher activity of HD-73 protein against cotton bollworm compared to HD-1 protein. In cases where the transgenic plants were damaged by the insects, the surviving larvae were delayed in their development by at least one instar.

[0114] Therefore, the increased expression obtained with the modified and synthetic genes is not limited to any one crop; tobacco, tomato and cotton calli and cotton plants all showed drastic increases in *B.t.k.* expression when the plants/calli were produced containing the modified or synthetic genes. Likewise, the utility of changes made to produce the modified and synthetic *B.t.k.* HD-1 gene is not limited to the HD-1 gene. The synthetic HD-73 gene in all three species also showed drastic increases in expression.

[0115] In summary, it has been demonstrated that: (1) the genetic changes made in the HD-1 modified gene lead to very significant increases in *B.t.k.* HD-1 expression; (2) production of a totally synthetic gene lead to a further five-fold increase in *B.t.k.* HD-1 expression; (3) the changes incorporated into the modified HD-1 gene accounted for the majority of the increased *B.t.k.* expression observed with the synthetic gene; (4) the increased expression was demonstrated in three different plants -- tobacco plants, tomato plants and cotton calli and cotton plants; (5) the increased expression as observed by Western analysis also correlated with similar increases in bioactivity, showing that the *B.t.k.* HD-1 proteins produced were comparably active; (6) when the method of the present invention used to design the synthetic HD-1 gene was employed to design a synthetic HD-73 gene it also was expressed at much higher levels in tobacco, tomato and cotton than the wild-type equivalent gene with consequent increases in bioactivity; (7) a fully synthetic full length *B.t.k.* gene was expressed at levels comparable to synthetic truncated genes.

Example 5 -- Synthetic *B.t. tenebrionis* Gene in Tobacco. Tomato and Potato

[0116] Referring to Figure 12, a synthetic gene encoding a Coleopteran active toxin is prepared by making the indicated changes in the wild-type gene of *B.t. tenebrionis* or de novo synthesis of the synthetic structural gene. The synthetic gene is inserted into an intermediate plant transformation vector such as pMON893: Plasmid pMON893 containing the synthetic *B.t.t.* gene is then inserted into a suitable disarmed *Agrobacterium* strain such as *A. tumefaciens* ACO.

Transformation and Regeneration of Potato

[0117] Sterile shoot cultures of Russet Burbank are maintained in vials containing 10 ml of PM medium (Murashige and Skoog (MS) inorganic salts, 30 g/l surcose, 0.17 g/l $NaH_2PO_4H_2O$, 0.4 mg/l thiamine-HCl, and 100 mg/l myo-inositol, solidified with 1 g/l Gelrite at pH 6.0). When shoots reached approximately 5 cm in length, stem internode segments of 7-10 mm are excised and smeared at the cut ends with a disarmed *Agrobacterium tumefaciens* vector containing the synthetic *B.t.t.* gene from a four day old plate culture. The stem explants are co-cultured for three days at 23°c on a sterile filter paper placed over 1.5 ml of a tobacco cell feeder layer overlaid on 1/10 P medium (1/10 strength MS inorganic salts and organic addenda without casein as in Jarret et al. (1980), 30 g/l surcose and 8.0 g/l agar). Following co-culture the explants are transferred to full strength P-1 medium for callus induction, composed of MS inorganic salts, organic additions as in Jarret et al. (1980) with the exception of casein, 3.0 mg/l benzyladenine (BA), and 0.01 mg/l naphthaleneacetic acid (NAA) (Jarret, et al., 1980). Carbenicillin (500 mg/l) is included to inhibit

bacterial growth, and 100 mg/l kanamycin is added to select for transformed cells. After four weeks the explants are transferred to medium of the same composition but with 0.3 mg/l gibberellic acid (GA3) replacing the BA and NAA (Jarret et al., 1981) to promote shoot formation. Shoots begin to develop approximately two weeks after transfer to shoot induction medium; these are excised and transferred to vials of PM medium for rooting. Shoots are tested for kanamycin resistance conferred by the enzyme neomycin phosphotransferase II, by placing a section of the stem onto callus induction medium containing MS organic and inorganic salts, 30 g/l surcrose, 2.25 mg/l BA, 0.186 mg/l NAA, 10 mg/l GA3 (Webb, et al., 1983) and 200 mg/l kanamycin to select for transformed cells.

[0118] The synthetic *B.t.t.* gene described in figure 12, was placed into a plant expression vector as descibed in example 5. The plasmid has the following characteristics; a synthetic BglII fragment having approximately 1800 base pairs was inserted into pMON893 in such a manner that the enhanced 35S promoter would express the *B.t.t.* gene. This construct, pMON1982, was used to transform both tobacco and tomato. Tobacco plants, selected as kanamycin resistant plants were screened with rabbit anti-*B.t.t.* antibody. Cross-reactive material was detected at levels predicted to be suitable to cause mortality to CPB. These target insects will not feed on tobacco, but the transgenic tobacco plants do demonstrate that the synthetic gene does improve expression of this protein to detectable levels.

[0119] Tomato plants with the pMON1982 construct were determined to produce *B.t.t.* protein at levels insecticidal to CPB. In initial studies, the leaves of four plants (5190, 5225, 5328 and 5133) showed little or no damage when exposed to CPB larvae (damage rating of 0-1 on a scale of 0 to 4 with 4 as no leaf remaining). Under these conditions the control leaves were completely eaten. Immunological analysis of these plants confirmed the presence of material cross-reactive with anti-*B.t.t.* antibody. Levels of protein expression in these plants were estimated at aproximately 1 to 5 ng of *B.t.t.* protein in 50 ug of total extractable protein. A total of 17 tomato plants (17 of 65 tested) have been identified which demonstrate protection of leaf tissue from CPB (rating of 0 or 1) and show good insect mortality.

[0120] Results similar to those seen in tobacco and tomato with pMON1982 were seen with pMON1984 in the same plant species. pMON1984 is identical to pMON1982 except that the synthetic protease inhibitor (CMTI) is fused upstream of the native proteolytic cleavage site. Levels of expression in tobacco were estimated to be similar to pMON1982, between 10-15 ng per 50ug of total soluble protein.

[0121] Tomato plants expressing pMON1984 have been identified which protect the leaves from ingestion by CPB. The damage rating was 0 with 100% insect mortality.

[0122] Potato was transformed as described in example 5 with a vector similar to pMON1982 containing the enhanced CaMV35S/synthetic *B.t.t.* gene. Leaves of potato plants transformed with this vector, were screened by CPB insect bioassay. Of the 35 plants tested, leaves from 4 plants, 16a, 13c, 13d, and 23a were totally protected when challenged. Insect bioassays with leaves from three other plants, 13e, la, and 13b, recorded damage levels of 1 on a scale of 0 to 4 with 4 being total devestation of the leaf material. Immunological analysis confirmed the presence of *B.t.t.* cross-reactive material in the leaf tissue. The level of *B.t.t.* protein in leaf tissue of plant 16a (damage rating of 0) was estimated at 20-50 ng of *B.t.t.* protein/50 ug of total soluble protein. The levels of *B.t.t.* protein seen in 16a tissue was consistent with its biological activity. Immunological analysis of 13e and 13b (tissue which scored 1 in damage rating) reveal less protein (5-10 ng/50 ug of total soluble protein) than in plant 16a. Cuttings of plant 16a were challenged with 50 to 200 eggs of CPB in a whole plant assay. Under these conditions 16a showed no damage and 100% mortality of insects while control potato plants were heavily damaged.

Example 6 – Synthetic *B.t.k.* P2 Protein Gene

[0123] The P2 protein is a distinct insecticidal protein produced by some strains of *B.t.* including *B.t.k.* HD-1. It is characterized by its activity against both lepidopteran and dipteran insects (Yamamoto and Iizuka, 1983). Genes encoding the P2 protein have been isolated and characterized (Donovan et al., 1988). The P2 proteins encoded by these genes are approximately 600 amino acids in length. These proteins share only limited homology with the lepidopteran specific P1 type proteins, such as the *B.t.k.* HD-1 and HD-73 proteins described in previous examples.

[0124] The P2 proteins have substantial activity against a variety of lepidopteran larvae including cabbage looper, tobacco hornworm and tobacco budworm. Because they are active against agronomically important insect pests, the P2 proteins are a desirable candidate in the production of insect tolerant transgenic plants either alone or in combination with the other *B.t.* toxins described in the above examples. In some plants, expression of the P2 protein alone might be sufficient to provide protection against damaging insects. In addition, the P2 proteins might provide protection against agronomically important dipteran pests. In other cases, expression of P2 together with the *B.t.k.* HD-1 or HD-73 protein might be preferred. The P2 proteins should provide at least an additive level of insecticidal activity when combined with the crystal protein toxin of *B.t.k.* HD-1 or HD-73, and the combination may even provide a synergistic activity. Although the mode of action of the P2 protein is unknown, its distinct amino acid sequence suggests that it functions differently from the *B.t.k.* HD-1 and HD-73 type of proteins. Production of two insect tolerance proteins with different modes of action in the same plant would minimize the potential for development of insect resistance to *B.t.* proteins in plants. The lack of substantial DNA homology between P2 genes and the HD-1 and HD-73 genes minimizes the po-

tential for recombination between multiple insect tolerance genes in the plant chromosome.

[0125] The genes encoding the P2 protein although distinct in sequence from the *B.t.k.* HD-1 and HD-73 genes share many common features with these genes. In particular, the P2 protein genes have a high A+T content (65%), multiple potential polyadenylation signal sequences (26) and numerous ATTTA sequences (10). Because of its overall similarity to the poorly expressed wild-type *B.t.k.* HD-1 and HD-73 genes, the same problems are expected in expression of the wild-type P2 gene as were encountered with the previous examples. Based on the above-described method for designing the synthetic *B.t.* genes, a synthetic P2 gene has been designed which gene should be expressed at adequate levels for protection in plants. A comparision of the wild-type and synthetic P2 genes is shown in Figure 13.

Example 7 -- Synthetic *B.t. Entomocidus* Gene

[0126] The *B.t. entomocidus* ("Btent") protein is a distinct insecticidal protein produced by some strains of *B.t.* bacteria. It is characterized by its high level of activity against some lepidopterans that are relatively insensitive to *B.t.k.* HD-1 and HD-73 such as Spodoptera species including beet armyworm (Visser et al., 1988). Genes encoding the Btent protein have been isolated and characterized (Honee et al, 1988). The Btent proteins encoded by these genes are approximately the same length as *B.t.k.* HD-1 and HD-73. These proteins share only 68% amino acid homology with the *B.t.k.* HD-1 and HD-73 proteins. It is likely that only the N-terminal half of the Btent protein is required for insecticidal activity as is the case for HD-1 and HD-73. Over the first 625 amino acids, Btent shares only 38% amino acid homology with HD-1 and HD-73.

[0127] Because of their higher activity against Spodoptera species that are relatively insensitive to HD-1 and HD-73, the Btent proteins are a desirable candidate for the production of insect tolerant transgenic plants either alone or in combination with the other *B.t.* toxins described in the above examples. In some plants production of Btent alone might be sufficient to control the-agronomically important pests. In other plants, the production of two distinct insect tolerance proteins would provide protection against a wider array of insects. Against those insects where both proteins are active, the combination of the *B.t.k.* HD-1 or HD-73 type protein plus the Btent protein should provide at least additive insecticidal efficacy, and may even provide a synergistic activity. In addition, because of its distinct amino acid sequence, the Btent protein may have a different mode of action than HD-1 or HD-73. Production of two insecticidal proteins in the same plant with different modes of action would minimize the potential for development of insect resistance to *B.t.* proteins in plants. The relative lack of DNA sequence homology with the *B.t.k.* type genes minimizes the potential for recombination between multiple insect tolerance genes in the plant chromosome.

[0128] The genes encoding the Btent protein although distinct in sequence from the *B.t.k.* HD-1 and HD-73 genes share many common features with these genes. In particular, the Btent protein genes have a high A+T content (62%), multiple potential polyadenylation signal sequences (39 in the full length coding sequence and 27 in the first 1875 nucleotides that is likely to encode the active toxic fragment) and numerous ATTTA sequences (16 in the full length coding sequence and 12 in the first 1875 nucleotides). Because of its overall similarity to the poorly expressed wild type *B.t.k.* HD-1 and HD-73 genes, the wild-type Btent genes are expected to exhibit similar problems in expression as were encountered with the wild-type HD-1 and HD-73 genes. Based on the above-described method used for designing the other synthetic *B.t.* genes, a synthetic Btent gene has been designed which gene should be expressed at adequate levels for protection in plants. A comparision of the wild type and synthetic Btent genes is shown in Figure 14.

Example 8 -- Synthetic *B.t.k.* Genes for Expression in Corn

[0129] High level expression of heterologous genes in corn cells has been shown to be enhanced by the presence of a corn gene intron (Callis et al., 1987). Typically these introns have been located in the 5' untranslated region of the chimeric gene. It has been shown that the CaMV35S promoter and the NOS 3' end function efficiently in the expression of heterologous genes in corn cells (Fromm et al., 1986).

[0130] Referring to Figure 15, a plant expression cassette vector (pMON744) was constructed that contains these sequences. Specifically the expression cassette contains the enhanced CaMV 35S promoter followed by intron 1 of the corn Adhl gene (Callis et al., 1987). This is followed by a multilinker cloning site for insertion of coding sequences; this multilinker contains a BglII site among others. Following the multilinker is the NOS 3' end. pMON744 also contains the selectable marker gene 35S/NPTII/NOS 3' for kanamycin selection of transgenic corn cells. In addition, pMON744 has an E. *coli* origin of replication and an ampicillin resistance gene for selection of the plasmid in E. *coli.*

[0131] Five *B.t.k.* coding sequences described in the previous examples were inserted into the BglII site of pMON744 for corn cell expression of *B.t.k.* The coding sequences inserted and resulting vectors were:

1. Wild type *B.t.k.* HD-1 from pMON9921 to make pMON8652.
2. Modified *B.t.k.* HD-1 from pMON5370 to make pMON8642.

3. Synthetic *B.t.k.* HD-1 from pMON5377 to make pMON8643.
4. Synthetic *B.t.k.* HD-73 from pMON5390 to make pMON8644.
5. Synthetic full length *B.t.k.* HD-73 from pMON10518 to make pMON10902.

[0132] pMON8652 (wild-type *B.t.k.* HD-1) was used to transform corn cell protoplasts and stably transformed kanamycin resistant callus was isolated. *B.t.k.* mRNA in the corn cells was analyzed by nuclease S1 protection and found to be present at a level comparable to that seen with the same wild-type coding sequence (pMON9921) in transgenic tomato plants.

[0133] pMON8652 and pMON8642 (modified HD-1) were used to transform corn cell protoplasts in a transient expression system. The level of *B.t.k.* mRNA was analyzed by nuclease S1 protection. The modified HD-1 gave rise to a several fold increase in *B.t.k.* mRNA compared to the wild-type coding sequence in the transiently transformed corn cells. This indicated that the modifications introduced into the *B.t.k.* HD-1 gene are capable of enhancing *B.t.k.* expression in monocot cells as was demonstrated for dicot plants and cells.

[0134] pMON8642 (modified HD-1) and pMON8643 (synthetic HD-1) were used to transform Black Mexican Sweet (BMS) corn cell protoplasts by PEG-mediated DNA uptake, and stably transformed corn callus was selected by growth on kanamycin containing plant growth medium. Individual callus colonies that were derived from single transformed cells were isolated and propagated separately on kanamycin containing medium.

[0135] To assess the expression of the *B.t.k.* genes in these cells, callus samples were tested for insect toxicity by bioassay against tobacco hornworm larvae. For each vector, 96 callus lines were tested by bioassay. Portions of each callus were placed on sterile water agar plates, and five neonate tobacco hornworm larvae were added and allowed to feed for 4 days. For pMON8643, 100% of the larvae died after feeding on 15 of the 96 calli and these calli showed little feeding damage. For pMON8642, only 1 of the 96 calli was toxic to the larvae. This showed that the *B.t.k.* gene was being expressed in these samples at insecticidal levels. The observation that significantly more calli containing pMON8643 were toxic than for pMON8642 showed that significantly higher levels of expression were obtained when the synthetic HD-1 coding sequence was contained in corn cells than when the modified HD-1 coding sequence was used, similar to the previous examples with dicot plants. A semiquantitative immunoassay showed that the pMON8643 toxic samples had significantly higher *B.t.k.* protein levels than the pMON8642 toxic sample.

[0136] The 16 callus samples that were toxic to tobacco hornworm were also tested for activity against European corn borer. European corn borer is approximately 40-fold less sensitive to the HD-1 gene product than is tobacco hornworm. Larvae of European corn borer were applied to the callus samples and allowed to feed for 4 days. Two of the 16 calli tested, both of which contained pMON8643 (synthetic HD-1), were toxic to European corn borer larvae.

[0137] To assess the expression of the *B.t.k.* genes in differentiated corn tissue, another method of DNA delivery was used. Young leaves were excised from corn plants, and DNA samples were delivered into the leaf tissue by microprojectile bombardment. In this system, the DNA on the microprojectiles is transiently expressed in the leaf cells after bombardment. Three DNA samples were used, and each DNA was tested in triplicate.

1. pMON744, the corn expression vector with no *B.t.k.* gene.
2. pMON8643 (synthetic HD-1).
3. pMON752, a corn expression vector for the GUS gene, no *B.t.k.* gene.

[0138] The leaves were incubated at room temperature for 24 hours. The pMON752 samples were stained with a substrate that allows visual detection of the GUS gene product. This analysis showed that over one hundred spots in each sample were expressing the GUS product and the the triplicate samples showed very similar levels of GUS expression. For the pMON744 and pMON8643 samples 5 larvae of tobacco hornworm were added to each leaf and allowed to feed for 48 hours. All three samples bombarded with pMON744 showed extensive feeding damage and no larval mortality. All three samples bombarded with pMON8643 showed no evidence of feeding damage and 100% larval mortality. The samples were also assayed for the presence of *B.t.k.* protein by a qualitative immunoassay. All of the pMON8643 samples had detectable *B.t.k.* protein. These results demonstrated that the the synthetic *B.t.k.* gene was expressed in differentiated corn plant tissue at insecticidal levels.

Example 9 -- Expression of Synthetic *B.t.* Genes with RUBISCO Small Subunit Promoters and Chloroplast Transit Peptides

[0139] The genes in plants encoding the small subunit of RUBISCO (SSU) are often highly expressed, light regulated and sometimes show tissue specificity. These expression properties are largely due to the promoter sequences of these genes. It has been possible to use SSU promoters to express heterologous genes in transformed plants. Typically a plant will contain multiple SSU genes, and the expression levels and tissue specificity of different SSU genes will be different. The SSU proteins are encoded in the nucleus and synthesized in the cytoplasm as precursors that contain

an N-terminal extension known as the chloroplast transit peptide (CTP). The CTP directs the precursor to the chloroplast and promotes the uptake of the SSU protein into the chloroplast. In this process, the CTP is cleaved from the SSU protein. These CTP sequences have been used to direct heterologous proteins into chloroplasts of transformed plants.

[0140] The SSU promoters might have several advantages for expression of *B.t.k.* genes in plants. Some SSU promoters are very highly expressed and could give rise to expression levels as high or higher than those observed with the CaMV35S promoter. The tissue distribution of expression from SSU promoters is different from that of the CaMV35S promoter, so for control of some insect pests, it may be advantageous to direct the expression of *B.t.k.* to those cells in which SSU is most highly expressed. For example, although relatively constitutive, in the leaf the CaMV35S promoter is more highly expressed in vascular tissue than in some other parts of the leaf, while most SSU promoters are most highly expressed in the mesophyll cells of the leaf. Some SSU promoters also are more highly tissue specific, so it could be possible to utilize a specific SSU promoter to express *B.t.k.* in only a subset of plant tissues, if for example B.t. expression in certain cells was found to be deleterious to those cells. For example, for control of Colorado potato beetle in potato, it may be advantageous to use SSU promoters to direct *B.t.t.* expression to the leaves but not to the edible tubers.

[0141] Utilizing SSU CTP sequences to localize *B.t.* proteins to the chloroplast might also be advantageous. Localization of the *B.t.* to the chloroplast could protect the protein from proteases found in the cytoplasm. This could stabilize the *B.t.* protein and lead to higher levels of accumulation of active protein. *B.t.* genes containing the CTP could be used in combination with the SSU promoter or with other promoters such as CaMV35S.

[0142] A variety of plant transformation vectors were constructed for the expression of *B.t.k.* genes utilizing SSU promoters and SSU CTPs. The promoters and CTPs utilized were from the petunia SSU11a gene described by Tumer et al. (1986) and from the *Arabidopsis* atslA gene (an SSU gene) described by Krebbers et al. (1988) and by Elionor et al. (1989). The petunia SSU11a promoter was contained on a DNA fragment that extended approximately 800 bp upstream of the SSU coding sequence. The *Arabidopsis* ats1A promoter was contained on a DNA fragment that extended approximately 1.8 kb upstream of the SSU coding sequence. At the upstream end convenient sites from the multilinker of pUC18 were used to move these promoters into plant transformation vectors such as pMON893. These promoter fragments extended to the start of the SSU coding sequence at which point an NcoI restriction site was engineered to allow insertion of the *B.t.* coding sequence, replacing the SSU coding sequence.

[0143] When SSU promoters were used in combination with their CTP, the DNA fragments extended through the coding sequence of the CTP and a small portion of the mature SSU coding sequence at which point an NcoI restriction site was engineered by standard techniques to allow the in frame fusion of *B.t.* coding sequences with the CTP. In particular, for the petunia SSU11a CTP, *B.t.* coding sequences were fused to the SSU sequence after amino acid 8 of the mature SSU sequence at which point the NcoI site was placed. The 8 amino acids of mature SSU sequence were included because preliminary in vitro chloroplast uptake experiments indicated that uptake was of *B.t.k.* was observed only if this segment of mature SSU was included. For the Arabidopsis ats1A CTP, the complete CTP was included plus 24 amino acids of mature SSU sequence plus the sequence gly-gly-arg-val-asn-cys-met-gln-ala-met, terminating in an NcoI site for *B.t.* fusion. This short sequence reiterates the native SSU CTP cleavage site (between the cys and met) plus a short segment surrounding the cleavage site. This sequence was included in order to insure proper uptake into chloroplasts. *B.t.* coding sequences were fused to this atslA CTP after the met codon. In vitro uptake experiments with this CTP construction and other (non-*B.t.*) coding sequences showed that this CTP did target proteins to the chloroplast.

[0144] When CTPs were used in combination with the CaMV 35S promoter, the same CTP segments were used. They were excised just upstream of the ATG start sites of the CTP by engineering of BglII sites, and placed downstream of the CaMV35S promoter in pMON893, as BglII to NcoI fragments. *B.t.* coding sequences were fused as described above.

[0145] The wild type *B.t.k.* HD-1 coding sequence of pMON9921 (see Figure 1) was fused to the ats1A promoter to make pMON1925 or the ats1A promoter plus CTP to make pMON1921. These vectors were used to transform tobacco plants, and the plants were screened for activity against tobacco hornworm. No toxic plants were recovered. This is surprising in light of the fact that toxic plants could be recovered, albeit at a low frequency, after transformation with pMON9921 in which the *B.t.k.* coding sequence was expressed from the enhanced CaMV35S, promoter in pMON893, and in light of the fact that Elionor et al. (1989) report that the atslA promoter itself is comparable in strength to the CaMV35S promoter and approximately 10-fold stronger when the CTP sequence is included. At least for the wild-type *B.t.k.* HD-1 coding sequence, this does not appear to be the case.

[0146] A variety of plant transformation vectors were constructed utilizing either the truncated synthetic . HD-73 coding sequence of Figure 4 or the full length *B.t.k.* HD-73 coding sequence of Figure 11. These are listed in the table below.

Table XV

Gene Constructs with CTPs			
Vector	Promoter	CTP	*B.t.k.* HD-73 Coding Sequence
pMON10806	En 35S	ats1A	truncated
pMON10814	En35S	SSU11a	full length
pMON10811	SSU11a	SSU11a	truncated
pMON10819	SSU11a	none	truncated
pMON10815	ats1A	none	truncated
pMON10817	ats1A	ats1A	truncated
pMON10821	En 35S	ats1A	truncated
pMON10822	En 35S	ats1A	full length
pMON10838	SSU11a	SSU11a	full length
pMON10839	ats1A	ats1A	full length

[0147] All of the above vectors were used to transform tobacco plants. For all of the vectors containing truncated *B. t.k.* genes, leaf tissue from these plants has been analyzed for toxicity to insects and *B.t.k.* protein levels by immunoassay. pMON10806, 10811, 10819 and 10821 produce levels of *B.t.k.* protein comparable to pMON5383 and pMON5390 which contain synthetic *B.t.k.* HD-73 coding sequences driven by the En 35S promoter itself with no CTP. These plants also have the insecticidal activity expected for the *B.t.k.* protein levels detected. For pMON10815 and pMON10817 (containing the atsIA promoter), the level of *B.t.k.* protein is about 5-fold higher than that found in plants containing pMON5383 or 5390. These plants also have higher insecticidal activity. Plants containing 10815 and 10817 contain up to 1% of their total soluble leaf protein as *B.t.k.* HD-73. This is the highest level of *B.t.k.* protein yet obtained with any of the synthetic genes.

[0148] This result is surprising in two respects. First, as noted above, the wild type coding sequences fused to the ats1A promoter and CTP did not show any evidence of higher levels of expression than for En 35S, and in fact had lower expression based on the absence of any insecticidal plants. Second, Elionor et al. (1989) show that for two other genes, the atsIA CTP can increase expression from the atsIA promoter by about 10-fold. For the synthetic *B.t.k.* HD-73 gene, there is no consistent increase seen by including the CTP over and above that seen for the atsIA promoter alone.

[0149] Tobacco plants containing the full length synthetic HD-73 fused to the SSU11A CTP and driven by the En 35S promoter produced levels of *B.t.k.* protein and insecticidal activity comparable to pMON1518 which contains does not include the CTP. In addition, for pMON10518 the *B.t.k.* protein extracted from plants was observed by gel electrophoresis to contain multiple forms less than full length, apparently due the cleavage of the C-terminal portion (not required for toxicity) in the cytoplasm. For pMON10814, the majority of the protein appeared to be intact full length indicating that the protein has been stabilized from proteolysis by targeting to the chloroplast.

Example 10 -- Targeting of *B.t.* Proteins to the Extracellular Space or Vacuole through the Use of Signal Peptides

[0150] The *B.t.* proteins produced from the synthetic genes described here are localized to the cytoplasm of the plant cell, and this cytoplasmic localization results in plants that are insecticidally effective. It may be advantageous for some purposes to direct the *B.t.* proteins to other compartments of the plant cell. Localizing *B.t.* proteins in compartments other than the cytoplasm may result in less exposure of the *B.t.* proteins to cytoplasmic proteases leading to greater accumulation of the protein yielding enhanced insecticidal activity. Extracellular localization could lead to more efficient exposure of certain insects to the *B.t.* proteins leading to greater efficacy. If a *B.t.* protein were found to be deleterious to plant cell function, then localization to a noncytoplasmic compartment could protect these cells from the . protein.

[0151] In plants as well as other eucaryotes, proteins that are destined to be localized either extracellularly or in several specific compartments are typically synthesized with an N-terminal amino acid extension known as the signal peptide. This signal peptide directs the protein to enter the compartmentalization pathway, and it is typically cleaved from the mature protein as an early step in compartmentalization. For an extracellular protein, the secretory pathway typically involves cotranslational insertion into the endoplasmic reticulum with cleavage of the signal peptide occuring at this stage. The mature protein then passes thru the Golgi body into vesicles that fuse with the plasma membrane thus releasing the protein into the extracellular space. Proteins destined for other compartments follow a similar pathway. For example, proteins that are destined for the endoplasmic reticulum or the Golgi body follow this scheme,' but they are specifically retained in the appropriate compartment. In plants, some proteins are also targeted to the vacuole,

another membrane bound compartment in the cytoplasam of many plant cells. Vacuole targeted proteins diverge from the above pathway at the Golgi body where they enter vesicles that fuse with the vacuole.

[0152] A common feature of this protein targeting is the signal peptide that initiates the compartmentalization process. Fusing a signal peptide to a protein will in many cases lead to the targeting of that protein to the endoplasmic reticulum. The efficiency of this step may depend on the sequence of the mature protein itself as well. The signals that direct a protein to a specific compartment rather than to the extracellular space are not as clearly defined. It appears that many of the signals that direct the protein to specific compartments are contained within the amino acid sequence of the mature protein. This has been shown for some vacuole targeted proteins, but it is not yet possible to define these sequences precisely. It appears that secretion into the extracellular space is the "default" pathway for a protein that contains a signal sequence but no other compartmentalization signals. Thus, a strategy to direct *B.t.* proteins out of the cytoplasm is to fuse the genes for synthetic *B.t.* genes to DNA sequences encoding known plant signal peptides. These fusion genes will give rise to *B.t.* proteins that enter the secretory pathway, and lead to extracellualar secretion or targeting to the vacuole or other compartments.

[0153] Signal sequences for several plant genes have been described. One such sequence is for the tobacco pathogenesis related protein PR1b described by Cornelissen et al. The PR1b protein is normally localized to the extracellular space. Another type of signal peptide is contained on seed storage proteins of legumes. These proteins are localized to the protein body of seeds, which is a vacuole like compartment found in seeds. A signal peptide DNA sequence for the beta subunit of the 7S storage protein of common bean (Phaseolus vulgaris), PvuB has been described by Doyle et al. Based on the published these published sequences, genes were synthesized by chemical synthesis of oligonucleotides that encoded the signal peptides for PR1b and PvuB. The synthetic genes for these signal peptides corresponded exactly to the reported DNA sequences. Just upstream of the translational intiation codon of each signal peptide a BamHI and BglII site were inserted with the BamHI site at the 5' end. This allowed the insertion of the signal peptide encoding segments into the BglII site of pMON893 for expression from the En 35S promoter. In some cases to achieve secretion or compartmentalization of heterologous proteins, it has proved necessary to include some amino acid sequence beyond the normal cleavage site of the signal peptide. This may be necessary to insure proper cleavage of the signal peptide. For PR1b the synthetic DNA sequence also included the first 10 amino acids of mature PR1b. For PvuB the synthetic DNA sequence included the first 13 amino acids of mature PvuB. Both synthetic signal peptide encoding segments ended with NcoI sites to allow fusion in frame to the methionine initiation codon of the synthetic B.t. genes.

[0154] Four vectors encoding synthetic *B.t.k.* HD-73 genes were constructed containing these signal peptides. The synthetic truncated HD-73 gene from pMON5383 was fused with the signal peptide sequence of PvuB and incorporated into pMON893 to create pMON10827. The synthetic truncated HD-73 gene from pMON5383 was also fused with the signal peptide sequence of PR1b to create pMON10824. The full length synthetic HD-73 gene from pMON10518 was fused with the signal peptide sequence of PvuB and incorporated into pMON893 to create pMON10828. The full length synthetic HD-73 gene from pMON10518 was also fused with the signal peptide sequence of PR1b and incorporated into pMON893 to create pMON10825.

[0155] These vectors were used to transform tobacco plants and the plants were assayed for expression of the *B.t. k.* protein by Western blot analysis and for insecticidal efficacy. pMON10824 and pMON10827 produced amounts of *B.t.k.* protein in leaf comparable to the truncated HD-73 vectors, pMON5383 and pMON5390. pMON10825 and pMON10828 produced full length *B.t.k.* protein in amounts comparable to pMON10518. In all cases, the plants were insecticidally active against tobacco hornworm.

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Claims

1. A method for modifying a wild-type structural gene sequence which encodes an insecticidal protein of *Bacillus thuringiensis* to enhance the expression of said protein in plants which comprises:

   a) identifying regions within said sequence with greater than four consecutive adenine or thymine nucleotides;

   b) modifying the regions of step (a) which have two or more polyadenylation signals within a ten base sequence to remove said signals while maintaining a gene sequence which encodes said protein; and

   c) modifying the 15-30 base regions surrounding the regions of step (a) to remove major plant polyadenylation signals, consecutive sequences containing more than one minor polyadenylation signal and consecutive sequences containing more than one ATTTA sequence while maintaining a gene sequence which encodes said protein.

2. A method for modifying a wild-type structural gene sequence which encodes an insecticidal protein of *Bacillus thuringiensis* to enhance the expression of said protein in plants which comprises:

   a) removing polyadenylation signals contained in said wild-type gene while retaining a sequence which encodes said protein; and

   b) removing ATTTA sequences contained in said wild-type gene while retaining a sequence which encodes said protein.

3. A method of claim 2 further comprising the removal of self-complementary sequences and replacement of such sequences with nonself-complementary DNA comprising plant preferred codons while retaining a structural gene sequence encoding said protein.

4. A method of claims 1 to 3 further comprising the use of plant preferred sequences in the removal of the polyadenylation signals and ATTTA sequences.

5. A method of claims 1 to 3 in which the plant polyadenylation signals are selected from the group consisting of AATAAA, AATAAT, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA and CATAAA.

6. A method for improving the expression of a heterologous gene in plants wherein said gene comprises a modified chimeric gene containing a promoter which functions in plant cells operably linked to a structural coding sequence and a 3' non-translated region containing a polyadenylation signal which functions in plants to cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural coding sequence encodes an insecticidal protein at least a portion of which was derived from a *Bacillus thuringiensis* protein, wherein said method comprises modifying said structural coding sequence so that said sequence has a DNA sequence which differs from the naturally occurring DNA sequence encoding said *Bacillus thuringiensis* protein and said structural coding sequence does not contain more than 5 consecutive nucleotides consisting of either adenine or thymine residues.

7. A method for improving the expression of a heterologous gene in plants wherein said gene comprises a modified chimeric gene containing a promoter which functions in plant cells operably linked to a structural coding sequence and a 3' non-translated region containing a polyadenylation signal which functions in plants to cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural coding sequence encodes an insecticidal protein at least a portion of which was derived from a *Bacillus thuringiensis* protein, wherein said method comprises modifying said structural coding sequence so that said sequence has a DNA sequence which differs from the naturally occurring DNA sequence encoding said *Bacillus thuringiensis* protein and has the following characteristics:

   said structural coding sequence has a region which is complementary to the following sequence:

```
GGCTTGATTCCTAGCGAACTCTTCGATTCTCTGGTTGATGAGCTGTTC
1   5    10   15   20   25   30   35   40   45
```

said region in said coding sequence having eliminated 2 AACCAA and 1 AATTAA sequence.

8. A method according to claim 7, wherein said structural coding sequence encodes an insecticidal protein at least a portion of which was derived from a *Bacillus thuringiensis kurstakis* HD-1.

9. A method according to claim 7 or 8, wherein the plant is a tobacco plant.

10. A modified chimeric gene containing a promoter which functions in plant cells operably linked to a structural coding sequence and a 3' non-translated region containing a polyadenylation signal which functions in plants to cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural coding sequence encodes an insecticidal protein at least a portion of which was derived from a *Bacillus thuringiensis* protein, wherein said structural coding sequence has a DNA sequence which differs from the naturally occurring DNA sequence encoding said *Bacillus thuringiensis* protein and is selected from:

A. A structural gene which encodes an insecticidal protein of *B.t.k.* HD-1 having the sequence:

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  1  ATGGCTATAGAAACTGGTTACACCCCAATCGATATTTCCT   40
 41  TGTCGCTAACGCAATTTCTTTTGAGTGAATTTGTTCCCGG   80
 81  TGCTGGATTTGTGTTAGGACTAGTTGATATTATCTGGGGA  120
121  ATTTTTGGTCCCTCTCAATGGGACGCATTTCTTGTACAAA  160
161  TTGAACAGCTCATCAACCAGAGAATCGAAGAGTTCGCTAG  200
201  GAATCAAGCCATTTCTAGATTAGAAGGACTAAGCAATCTT  240
241  TATCAAATTTACGCAGAATCTTTTAGAGAGTGGGAAGCAG  280
281  ATCCTACTAATCCAGCATTAAGAGAAGAGATGCGTATTCA  320
321  ATTCAATGACATGAACAGTGCCCTTACAACCGCTATTCCT  360
361  CTTTTTGCAGTTCAAAATTATCAAGTTCCTCTCCTCTCCG  400
401  TGTACGTTCAAGCTGCCAACCTCCACCTCTCAGTTTTGAG  440
441  AGATGTTTCAGTGTTTGGACAAAGGTGGGGATTTGATGCC  480
481  GCGACTATCAATAGTCGTTATAATGATTTAACTAGGCTTA  520
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521   TTGGCAACTATACAGATCATGCTGTACGCTGGTACAATAC   560
561   GGGATTAGAGCGTGTATGGGGACCGGATTCTAGAGATTGG   600
601   ATCAGGTACAACCAGTTCAGAAGAGAGCTTACACTAACTG   640
641   TATTAGATATCGTTTCTCTATTTCCGAACTATGATAGTAG   680
681   AACGTATCCAATTCGAACAGTTTCCCAATTAACAAGAGAA   720
721   ATTTATACAAACCCAGTATTAGAAAATTTTGATGGTAGTT   760
761   TTCGAGGCTCGGCTCAGGGCATAGAAGGAAGTATTAGGAG   800
801   TCCACATTTGATGGATATACTTAATAGTATAACCATCTAT   840
841   ACGGATGCTCATAGAGGAGAATACTACTGGTCCGGTCACC   880
881   AGATCATGGCTTCTCCTGTAGGGTTTTCGGGGCCAGAATT   920
921   CACTTTTCCGCTATATGGAACTATGGGAAATGCAGCTCCA   960
961   CAACAACGTATTGTTGCTCAACTAGGTCAGGGCGTGTATA   1000
1001  GAACATTATCGTCCACCTTATATAGAAGACCTTTTAACAT   1040
1041  CGGGATCAACAACCAACAACTATCTGTTCTTGACGGGACA   1080
1081  GAATTTGCTTATGGAACCTCCTCAAATTTGCCATCCGCTG   1120
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1121 TATACAGAAAAAGCGGAACGGTAGATTCGCTGGATGAAAT 1160

1161 ACCGCCACAGAATAACAACGTGCCACCTAGGCAAGGATTT 1200

1201 AGTCATCGATTAAGCCATGTTTCAATGTTTCGTTCAGGCT 1240

1241 TTAGTAATAGTAGTGTAAGTATAATAAGAGCTCCTATGTT 1280

1281 CTCTTGGATACATCGTAGTGCTGAGTTCAACAACATCATC 1320

1321 CCTTCATCACAAATCACCCAAATCCCACTCACCAAGTCTA 1360

1361 CTAATCTTGGCTCTGGAACTTCTGTCGTTAAAGGACCAGG 1400

1401 ATTTACAGGAGGAGATATTCTTCGAAGAACTTCACCTGGC 1440

1441 CAGATTTCAACCTTAAGAGTAAATATTACTGCACCATTAT 1480

1481 CACAAAGATATCGGGTAAGAATTCGCTACGCTTCTACCAC 1520

1521 AAACCTTCAGTTCCACACATCAATTGACGGAAGACCTATT 1560

1561 AATCAGGGGAATTTTTCAGCAACTATGAGTAGTGGGAGTA 1600

1601 ATTTACAGTCCGGAAGCTTTAGGACTGTAGGTTTTACTAC 1640

1641 TCCGTTTAACTTTTCAAATGGATCAAGTGTATTTACGTTA 1680

1681 AGTGCTCATGTCTTCAATTCAGGCAATGAAGTTTATATAG 1720

1721 ATCGAATTGAATTTGTTCCGGCA 1743;

B. A structural gene which encodes an insecticidal protein of *B.t.k.* HD-73 having the sequence:

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  1 ATGGCCATTGAAACCGGTTACACTCCCATCGACATCTCCT  40
 41 TGTCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGG  80
 81 TGCTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGT 120
121 ATCTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAA 160
161 TTGAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAG 200
201 GAACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTC 240
241 TACCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCG 280
281 ATCCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCA 320
321 ATTCAACGACATGAACAGCGCCTTGACCACAGCTATCCCA 360
361 TTGTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCG 400
401 TGTACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCG 440
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 441  AGACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCT   480
 481  GCAACCATCAATAGCCGTTACAACGACCTTACTAGGCTGA   520
 521  TTGGAAACTACACCGACCACGCTGTTCGTTGGTACAACAC   560
 561  TGGCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGG   600
 601  ATTAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAG   640
 641  TTTTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAG   680
 681  AACCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAA   720
 721  ATCTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCT   760
 761  TCCGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAG   800
 801  CCCACACTTGATGGACATCTTGAACAGCATAACTATCTAC   840
 841  ACCGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACC   880
 881  AGATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTT   920
 921  TACCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCA   960
 961  CAACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACA  1000
1001  GAACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATAT  1040
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1041  CGGTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACA  1080

1081  GAGTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTG  1120

1121  TTTACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAAT  1160

1161  CCCACCACAGAACAACAATGTGCCACCCAGGCAAGGATTC  1200

1201  TCCCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGAT  1240

1241  TCAGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTT  1280

1281  CTCTTGGATACACCGTAGTGCTGAGTTCAACAACATCATC  1320

1321  GCATCCGATAGTATTACTCAAATCCCTGCAGTGAAGGGAA  1360

1361  ACTTTCTCTTCAACGGTTCTGTCATTTCAGGACCAGGATT  1400

1401  CACTGGTGGAGACCTCGTTAGACTCAACAGCAGTGGAAAT  1440

1441  AACATTCAGAATAGAGGGTATATTGAAGTTCCAATTCACT  1480

1481  TCCCATCCACATCTACCAGATATAGAGTTCGTGTGAGGTA  1520

1521  TGCTTCTGTGACCCCTATTCACCTCAACGTTAATTGGGGT  1560

1561  AATTCATCCATCTTCTCCAATACAGTTCCAGCTACAGCTA  1600

1601  CCTCCTTGGATAATCTCCAATCCAGCGATTTCGGTTACTT  1640
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1641 TGAAAGTGCCAATGCTTTTACATCTTCACTCGGTAACATC 1680

1681 GTGGGTGTTAGAAACTTTAGTGGGACTGCAGGAGTGATTA 1720

1721 TCGACAGATTCGAGTTCATTCCAGTTACTGCAACACTCGA 1760

1761 GGCTGAG 1767.

C. A structural gene encoding a insecticidal protein of *B.t.k.* HD-1 having the sequence:

1 ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA 40

41 ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA 80

81 ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG 120

121 TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG 160

161 CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTAT 200

201 CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT 240

241 GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA 280

281 ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA 320

321 CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT 360

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361  CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT  400
401  TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT  440
441  GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG  480
481  TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG  520
521  ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC  560
561  AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT  600
601  GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG  640
641  GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT  680
681  TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT  720
721  TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA  760
761  CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT  800
801  CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC  840
841  CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC  880
881  CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC  920
921  CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG  960
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 961  ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA  1000
1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA  1040
1041  ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA  1080
1081  ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG  1120
1121  GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA  1160
1161  GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT  1200
1201  TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC  1240
1241  CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC  1280
1281  CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC  1320
1321  AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT  1360
1361  CATGGATTCATCGTAGTGCTGAGTTCAACAATATCATTCC  1400
1401  TTCCTCTCAAATCACCCAAATCCCATTGACCAAGTCTACT  1440
1441  AACCTTGGATCTGGAACTTCTGTCGTGAAAGGACCAGGCT  1480
1481  TCACAGGAGGTGATATTCTTAGAAGAACTTCTCCTGGCCA  1520
1521  GATTAGCACCCTCAGAGTTAACATCACTGCACCACTTTCT  1560
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1561  CAAAGATATCGTGTCAGGATTCGTTACGCATCTACCACTA  1600

1601  ACTTGCAATTCCACACCTCCATCGACGGAAGGCCTATCAA  1640

1641  TCAGGGTAACTTCTCCGCAACCATGTCAAGCGGCAGCAAC  1680

1681  TTGCAATCCGGCAGCTTCAGAACCGTCGGTTTCACTACTC  1720

1721  CTTTCAACTTCTCTAACGGATCAAGCGTTTTCACCCTTAG  1760

1761  CGCTCATGTGTTCAATTCTGGCAATGAAGTGTACATTGAC  1800

1801  CGTATTGAGTTTGTGCCTGCCGAAGTTACCTTCGAGGCTG  1840

1841  AGTAC  1845.
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D. A structural gene encoding an insecticidal protein derived from *B.t.k.* HD-73 having the sequence:

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  1  ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA  40

 41  ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA  80

 81  ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG  120

121  TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG  160

161  CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGTAT  200
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201  CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT  240
241  GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA  280
281  ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA  320
321  CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT  360
361  CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT  400
401  TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT  440
441  GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG  480
481  TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG  520
521  ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC  560
561  AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT  600
601  GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG  640
641  GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT  680
681  TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT  720
721  TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA  760
761  CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT  800
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 801  CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC   840
 841  CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC   880
 881  CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC   920
 921  CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG   960
 961  ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA  1000
1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA  1040
1041  ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA  1080
1081  ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG  1120
1121  GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA  1160
1161  GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT  1200
1201  TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC  1240
1241  CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC  1280
1281  CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC  1320
1321  AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT  1360
1361  CTTGGATACACCGTAGTGCTGAGTTCAACAACATCATCGC  1400
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1401 ATCCGATAGTATTACTCAAATCCCTGCAGTGAAGGGAAAC 1440

1441 TTTCTCTTCAACGGTTCTGTCATTTCAGGACCAGGATTCA 1480

1481 CTGGTGGAGACCTCGTTAGACTCAACAGCAGTGGAAATAA 1520

1521 CATTCAGAATAGAGGGTATATTGAAGTTCCAATTCACTTC 1560

1561 CCATCCACATCTACCAGATATAGAGTTCGTGTGAGGTATG 1600

1601 CTTCTGTGACCCCTATTCACCTCAACGTTAATTGGGGTAA 1640

1641 TTCATCCATCTTCTCCAATACAGTTCCAGCTACAGCTACC 1680

1681 TCCTTGGATAATCTCCAATCCAGCGATTTCGGTTACTTTG 1720

1721 AAAGTGCCAATGCTTTTACATCTTCACTCGGTAACATCGT 1760

1761 GGGTGTTAGAAACTTTAGTGGGACTGCAGGAGTGATTATC 1800

1801 GACAGATTCGAGTTCATTCCAGTTACTGCAACACTCGAGG 1840

1841 CTGAATATAATCTGGAAAGAGCGCAGAAGGCGGTAATGCG 1880

1881 CTGTTTACGTCTACAAACCAGCTTGGACTCAAGACAAATG 1920.

1921 G 1921;

E. A structural gene encoding the full-length insecticidal protein of *B.t.k.* HD-73 having the sequence:

1 ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA 40

41 ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA 80

81 ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG 120

121 TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG 160

161 CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTAT 200

201 CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT 240

241 GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA 280

281 ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA 320

321 CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT 360

361 CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT 400

401 TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT 440

441 GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG 480

481 TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG 520

521 ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC 560

561 AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT 600

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 601  GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG   640
 641  GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT   680
 681  TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT   720
 721  TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA   760
 761  CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT   800
 801  CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC   840
 841  CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC   880
 881  CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC   920
 921  CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG   960
 961  ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA  1000
1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA  1040
1041  ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA  1080
1081  ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG  1120
1121  GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA  1160
1161  GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT  1200
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1201 TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC 1240

1241 CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC 1280

1281 CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC 1320

1321 AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT 1360

1361 CTTGGATACACCGTAGTGCTGAGTTCAACAACATCATCGC 1400

1401 ATCCGATAGTATTACTCAAATCCCTGCAGTGAAGGGAAAC 1440

1441 TTTCTCTTCAACGGTTCTGTCATTTCAGGACCAGGATTCA 1480

1481 CTGGTGGAGACCTCGTTAGACTCAACAGCAGTGGAAATAA 1520

1521 CATTCAGAATAGAGGGTATATTGAAGTTCCAATTCACTTC 1560

1561 CCATCCACATCTACCAGATATAGAGTTCGTGTGAGGTATG 1600

1601 CTTCTGTGACCCCTATTCACCTCAACGTTAATTGGGGTAA 1640

1641 TTCATCCATCTTCTCCAATACAGTTCCAGCTACAGCTACC 1680

1681 TCCTTGGATAATCTCCAATCCAGCGATTTCGGTTACTTTG 1720

1721 AAAGTGCCAATGCTTTTACATCTTCACTCGGTAACATCGT 1760

1761 GGGTGTTAGAAACTTTAGTGGGACTGCAGGAGTGATTATC 1800

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1801  GACAGATTCGAGTTCATTCCAGTTACTGCAACACTCGAGG  1840
1841  CTGAATATAATCTGGAAAGAGCGCAGAAGGCGGTGAATGC  1880
1881  GCTGTTTACGTCTACAAACCAGCTCGGCCTCAAGACCAAT  1920
1921  GTGACGGATTATCATATTGATCAAGTGTCCAACTTGGTGA  1960
1961  CCTACCTCAGCGATGAGTTCTGTCTGGATGAAAAGCGAGA  2000
2001  ATTGTCCGAGAAAGTCAAACATGCGAAGCGACTCAGTGAT  2040
2041  GAACGCAATTTACTCCAAGATTCAAATTTCAAAGACATTA  2080
2081  ATAGGCAACCAGAACGTGGGTGGGGCGGAAGTACAGGGAT  2120
2121  TACCATCCAGGGAGGTGACGACGTGTTCAAGGAGAACTAC  2160
2161  GTCACACTATCAGGTACCTTTGATGAGTGCTATCCAACAT  2200
2201  ACCTCTACCAGAAGATCGACGAGTCCAAGTTGAAAGCCTT  2240
2241  TACCCGTTATCAATTAAGAGGGTATATCGAAGATAGTCAA  2280
2281  GACCTCGAGATCTACCTCATCCGCTACAATGCAAAACATG  2320
2321  AAACAGTAAATGTGCCAGGTACGGGTTCCTTATGGCCGCT  2360
2361  TTCAGCCCAAAGTCCAATCGGAAAGTGTGGAGAGCCGAAT  2400
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2401  CGATGCGCGCCACACCTTGAATGGAATCCTGACTTAGATT  2440
2441  GTTCGTGTAGGGATGGAGAAAAGTGTGCCCATCATTCGCA  2480
2481  TCATTTCTCCTTAGACATTGATGTAGGATGTACAGACTTA  2520
2521  AATGAGGACCTAGGTGTATGGGTGATCTTTAAGATTAAGA  2560
2561  CGCAAGATGGGCACGCAAGACTAGGGAATCTAGAGTTTCT  2600
2601  CGAAGAGAAACCATTAGTAGGAGAAGCGCTAGCTCGTGTG  2640
2641  AAAAGAGCGGAGAAAAAATGGAGAGACAAACGTGAGAAGT  2680
2681  TGGAATGGGAGACCAACATCGTCTACAAAGAGGCAAAAGA  2720
2721  ATCTGTAGATGCTTTATTTGTAAACTCTCAATATGATCAA  2760
2761  TTACAAGCGGATACGAATATTGCCATGATTCATGCGGCAG  2800
2801  ATAAACGTGTTCATAGCATTCGAGAAGCTTATCTGCCTGA  2840
2841  GCTGTCTGTGATTCCGGGTGTCAATGCGGCTATTTTTGAA  2880
2881  GAATTAGAAGGGCGTATTTTCACTGCATTCTCCCTCTACG  2920
2921  ATGCCAGAAACGTCATCAAGAACGGTGACTTCAACAATGG  2960
2961  CTTATCCTGCTGGAACGTGAAAGGGCATGTAGATGTAGAA  3000
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3001	GAACAAAACAACCAACGTTCGGTCCTTGTTGTTCCGGAAT	3040
3041	GGGAAGCAGAAGTGTCACAAGAAGTTCGTGTCTGTCCGGG	3080
3081	TCGTGGCTATATCCTTCGTGTCACAGCGTACAAGGAGGGA	3120
3121	TATGGAGAAGGTTGCGTAACCATTCATGAGATCGAGAACA	3160
3161	ATACAGACGAACTGAAGTTTAGCAACTGCGTAGAAGAGGA	3200
3201	AATCTATCCAAATAACACGGTAACGTGTAATGATTATACT	3240
3241	GTAAATCAAGAAGAATACGGAGGTGCGTACACTTCTCGTA	3280
3281	ATCGAGGATATAACGAAGCTCCTTCCGTACCAGCTGATTA	3320
3321	TGCGTCAGTCTATGAAGAAAAATCGTATACAGATGGACGA	3360
3361	AGAGAGAATCCTTGTGAATTTAACAGAGGGTATAGGGATT	3400
3401	ACACGCCACTACCAGTTGGTTATGTGACAAAAGAATTAGA	3440
3441	ATACTTCCCAGAAACCGATAAGGTATGGATTGAGATTGGA	3480
3481	GAAACGGAAGGAACATTTATCGTGGACAGCGTGGAATTAC	3520
3521	TCCTTATGGAGGAA 3534.	

F. A structural gene encoding a full-length insecticidal protein of *B.t.k.* HD-73 having the sequence:

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  1  ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA   40
 41  ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA   80
 81  ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG  120
121  TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG  160
161  CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTAT  200
201  CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT  240
241  GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA  280
281  ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA  320
321  CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT  360
361  CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT  400
401  TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT  440
441  GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG  480
481  TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG  520
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 521  ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC   560
 561  AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT   600
 601  GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG   640
 641  GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT   680
 681  TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT   720
 721  TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA   760
 761  CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT   800
 801  CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC   840
 841  CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC   880
 881  CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC   920
 921  CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG   960
 961  ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA  1000
1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA  1040
1041  ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA  1080
1081  ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG  1120
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1121  GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA  1160
1161  GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT  1200
1201  TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC  1240
1241  CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC  1280
1281  CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC  1320
1321  AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT  1360
1361  CTTGGATACACCGTAGTGCTGAGTTCAACAACATCATCGC  1400
1401  ATCCGATAGTATTACTCAAATCCCTGCAGTGAAGGGAAAC  1440
1441  TTTCTCTTCAACGGTTCTGTCATTTCAGGACCAGGATTCA  1480
1481  CTGGTGGAGACCTCGTTAGACTCAACAGCAGTGGAAATAA  1520
1521  CATTCAGAATAGAGGGTATATTGAAGTTCCAATTCACTTC  1560
1561  CCATCCACATCTACCAGATATAGAGTTCGTGTGAGGTATG  1600
1601  CTTCTGTGACCCCTATTCACCTCAACGTTAATTGGGGTAA  1640
1641  TTCATCCATCTTCTCCAATACAGTTCCAGCTACAGCTACC  1680
1681  TCCTTGGATAATCTCCAATCCAGCGATTTCGGTTACTTTG  1720
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1721  AAAGTGCCAATGCTTTTACATCTTCACTCGGTAACATCGT  1760
1761  GGGTGTTAGAAACTTTAGTGGGACTGCAGGAGTGATTATC  1800
1801  GACAGATTCGAGTTCATTCCAGTTACTGCAACACTCGAGG  1840
1841  CTGAATATAATCTGGAAAGAGCGCAGAAGGCGGTGAATGC  1880
1881  GCTGTTTACGTCTACAAACCAACTAGGGCTAAAAACAAAT  1920
1921  GTAACGGATTATCATATTGATCAAGTGTCCAATTTAGTTA  1960
1961  CGTATTTATCGGATGAATTTTGTCTGGATGAAAAGCGAGA  2000
2001  ATTGTCCGAGAAAGTCAAACATGCGAAGCGACTCAGTGAT  2040
2041  GAACGCAATTTACTCCAAGATTCAAATTTCAAAGACATTA  2080
2081  ATAGGCAACCAGAACGTGGGTGGGGCGGAAGTACAGGGAT  2120
2121  TACCATCCAAGGAGGGGATGACGTATTTAAAGAAAATTAC  2160
2161  GTCACACTATCAGGTACCTTTGATGAGTGCTATCCAACAT  2200
2201  ATTTGTATCAAAAAATCGATGAATCAAAATTAAAAGCCTT  2240
2241  TACCCGTTATCAATTAAGAGGGTATATCGAAGATAGTCAA  2280
2281  GACTTAGAAATCTATTTAATTCGCTACAATGCAAAACATG  2320
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2321  AAACAGTAAATGTGCCAGGTACGGGTTCCTTATGGCCGCT  2360

2361  TTCAGCCCAAAGTCCAATCGGAAAGTGTGGAGAGCCGAAT  2400

2401  CGATGCGCGCCACACCTTGAATGGAATCCTGACTTAGATT  2440

2441  GTTCGTGTAGGGATGGAGAAAAGTGTGCCCATCATTCGCA  2480

2481  TCATTTCTCCTTAGACATTGATGTAGGATGTACAGACTTA  2520

2521  AATGAGGACCTAGGTGTATGGGTGATCTTTAAGATTAAGA  2560

2561  CGCAAGATGGGCACGCAAGACTAGGGAATCTAGAGTTTCT  2600

2601  CGAAGAGAAACCATTAGTAGGAGAAGCGCTAGCTCGTGTG  2640

2641  AAAAGAGCGGAGAAAAAATGGAGAGACAAACGTGAAAAAT  2680

2681  TGGAATGGGAAACAAATATCGTTTATAAAGAGGCAAAAGA  2720

2721  ATCTGTAGATGCTTTATTTGTAAACTCTCAATATGATCAA  2760

2761  TTACAAGCGGATACGAATATTGCCATGATTCATGCGGCAG  2800

2801  ATAAACGTGTTCATAGCATTCGAGAAGCTTATCTGCCTGA  2840

2841  GCTGTCTGTGATTCCGGGTGTCAATGCGGCTATTTTTGAA  2880

2881  GAATTAGAAGGGCGTATTTTCACTGCATTCTCCCTATATG  2920
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2921  ATGCGAGAAATGTCATTAAAAATGGTGATTTTAATAATGG  2960
2961  CTTATCCTGCTGGAACGTGAAAGGGCATGTAGATGTAGAA  3000
3001  GAACAAAACAACCAACGTTCGGTCCTTGTTGTTCCGGAAT  3040
3041  GGGAAGCAGAAGTGTCACAAGAAGTTCGTGTCTGTCCGGG  3080
3081  TCGTGGCTATATCCTTCGTGTCACAGCGTACAAGGAGGGA  3120
3121  TATGGAGAAGGTTGCGTAACCATTCATGAGATCGAGAACA  3160
3161  ATACAGACGAACTGAAGTTTAGCAACTGCGTAGAAGAGGA  3200
3201  AATCTATCCAAATAACACGGTAACGTGTAATGATTATACT  3240
3241  GTAAATCAAGAAGAATACGGAGGTGCGTACACTTCTCGTA  3280
3281  ATCGAGGATATAACGAAGCTCCTTCCGTACCAGCTGATTA  3320
3321  TGCGTCAGTCTATGAAGAAAAATCGTATACAGATGGACGA  3360
3361  AGAGAGAATCCTTGTGAATTTAACAGAGGGTATAGGGATT  3400
3401  ACACGCCACTACCAGTTGGTTATGTGACAAAAGAATTAGA  3440
3441  ATACTTCCCAGAAACCGATAAGGTATGGATTGAGATTGGA  3480

3481  GAAACGGAAGGAACATTTATCGTGGACAGCGTGGAATTAC  3520
3521  TCCTTATGGAGGAA  3534.
```

G. A structural gene encoding a full-length insecticidal protein of *B.t.k.* HD-73 having the sequence:

```
  1  ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA  40
 41  ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA  80
 81  ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG 120
121  TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG 160
161  CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGTAT  200
201  CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT 240
241  GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA 280
281  ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA 320
321  CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT 360
361  CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT 400
401  TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT 440
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441   GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG   480
481   TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG   520
521   ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC   560
561   AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT   600
601   GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG   640
641   GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT   680
681   TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT   720
721   TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA   760
761   CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT   800
801   CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC   840
841   CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC   880
881   CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC   920
921   CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG   960
961   ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA   1000
1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA   1040
```

1041 ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA 1080

1081 ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG 1120

1121 GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA 1160

1161 GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT 1200

1201 TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC 1240

1241 CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC 1280

1281 CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC 1320

1321 AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT 1360

1361 CTTGGATACACCGTAGTGCTGAGTTCAACAACATCATCGC 1400

1401 ATCCGATAGTATTACTCAAATCCCTGCAGTGAAGGGAAAC 1440

1441 TTTCTCTTCAACGGTTCTGTCATTTCAGGACCAGGATTCA 1480

1481 CTGGTGGAGACCTCGTTAGACTCAACAGCAGTGGAAATAA 1520

1521 CATTCAGAATAGAGGGTATATTGAAGTTCCAATTCACTTC 1560

1561 CCATCCACATCTACCAGATATAGAGTTCGTGTGAGGTATG 1600

1601 CTTCTGTGACCCCTATTCACCTCAACGTTAATTGGGGTAA 1640

```
1641  TTCATCCATCTTCTCCAATACAGTTCCAGCTACAGCTACC  1680
1681  TCCTTGGATAATCTCCAATCCAGCGATTTCGGTTACTTTG  1720
1721  AAAGTGCCAATGCTTTTACATCTTCACTCGGTAACATCGT  1760
1761  GGGTGTTAGAAACTTTAGTGGGACTGCAGGAGTGATTATC  1800
1801  GACAGATTCGAGTTCATTCCAGTTACTGCAACACTCGAGG  1840
1841  CTGAGTACAACCTTGAGAGAGCCCAGAAGGCTGTGAACGC  1880
1881  CCTCTTTACCTCCACCAATCAGCTTGGCTTGAAAACTAAC  1920
1921  GTTACTGACTATCACATTGACCAAGTGTCCAACTTGGTCA  1960
1961  CCTACCTTAGCGATGAGTTCTGCCTCGACGAGAAGCGTGA  2000
2001  ACTCTCCGAGAAAGTTAAACACGCCAAGCGTCTCAGCGAC  2040
2041  GAGAGGAATCTCTTGCAAGACTCCAACTTCAAAGACATCA  2080
2081  ACAGGCAGCCAGAACGTGGTTGGGGTGGAAGCACCGGGAT  2120
2121  CACCATCCAAGGAGGCGACGATGTGTTCAAGGAGAACTAC  2160
2161  GTCACCCTCTCCGGAACTTTCGACGAGTGCTACCCTACCT  2200
2201  ACTTGTACCAGAAGATCGATGAGTCCAAACTCAAAGCCTT  2240
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2241  CACCAGGTATCAACTTAGAGGCTACATCGAAGACAGCCAA  2280
2281  GACCTTGAAATCTACTCGATCAGGTACAATGCCAAGCACG  2320
2321  AGACCGTGAATGTCCCAGGTACTGGTTCCCTCTGGCCACT  2360
2361  TTCTGCCCAATCTCCCATTGGGAAGTGTGGAGAGCCTAAC  2400
2401  AGATGCGCTCCACACCTTGAGTGGAATCCTGACTTGGACT  2440
2441  GCTCCTGCAGGGATGGCGAGAAGTGTGCCCACCATTCTCA  2480
2481  TCACTTCTCCTTGGACATCGATGTGGGATGTACTGACCTG  2520
2521  AATGAGGACCTCGGAGTCTGGGTCATCTTCAAGATCAAGA  2560
2561  CCCAAGACGGACACGCAAGACTTGGCAACCTTGAGTTTCT  2600
2601  CGAAGAGAAACCATTGGTCGGTGAAGCTCTCGCTCGTGTG  2640
2641  AAGAGAGCAGAGAAGAAGTGGAGGGACAAACGTGAGAAAC  2680
2681  TCGAATGGGAAACTAACATCGTTTACAAGGAGGCCAAAGA  2720
2721  GTCCGTGGATGCTTTGTTCGTGAACTCCCAATATGATCAG  2760
2761  TTGCAAGCCGACACCAACATCGCCATGATCCACGCCGCAG  2800
2801  ACAAACGTGTGCACAGCATTCGTGAGGCTTACTTGCCTGA  2840
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2841  GTTGTCCGTGATCCCTGGTGTGAACGCTGCCATCTTCGAG  2880
2881  GAACTTGAGGGACGTATCTTTACCGCATTCTCCTTGTACG  2920
2921  ATGCCAGAAACGTCATCAAGAACGGTGACTTCAACAATGG  2960
2961  CCTCAGCTGCTGGAATGTGAAAGGTCATGTGGACGTGGAG  3000
3001  GAACAGAACAATCAGCGTTCCGTCCTGGTTGTGCCTGAGT  3040
3041  GGGAAGCTGAAGTGTCCCAAGAGGTTAGAGTCTGTCCAGG  3080
3081  TAGAGGCTACATTCTCCGTGTGACCGCTTACAAGGAGGGA  3120
3121  TACGGTGAGGGTTGCGTGACCATCCACGAGATCGAGAACA  3160
3161  ACACCGACGAGCTTAAGTTCTCCAACTGCGTCGAGGAAGA  3200
3201  AATCTATCCCAACAACACCGTTACTTGCAACGACTACACT  3240
3241  GTGAATCAGGAAGAGTACGGAGGTGCCTACACTAGCCGTA  3280
3281  ACAGAGGTTACAACGAAGCTCCTTCCGTTCCTGCTGACTA  3320
3321  TGCCTCCGTGTACGAGGAGAAATCCTACACAGATGGCAGA  3360
3361  CGTGAGAACCCTTGCGAGTTCAACAGAGGTTACAGGGACT  3400
3401  ACACACCACTTCCAGTTGGCTATGTTACCAAGGAGCTTGA  3440
```

```
3441  GTACTTTCCTGAGACCGACAAAGTGTGGATCGAGATCGGT  3480

3481  GAAACCGAGGGAACCTTCATCGTGGACAGCGTGGAGCTTC  3520

3521  TCTTGATGGAGGAA  3534.
```

H. A structural gene which encodes an insecticidal protein of *B.t.t.* having the sequence:

```
  1  ATGACTGCAGACAACAACACCGAAGCCCTCGACAGTTCTA   40

 41  CCACTAAGGATGTTATCCAGAAGGGTATCTCCGTTGTGGG   80

 81  AGACCTCTTGGGCGTGGTTGGATTTCCCTTCGGTGGAGCC  120

121  CTCGTGAGCTTCTATACAAACTTTCTCAACACCATTTGGC  160

161  CAAGCGAGGACCCTTGGAAAGCATTCATGGAGCAAGTTGA  200

201  AGCTCTTATGGATCAGAAGATTGCAGATTATGCCAAGAAC  240

241  AAGGCTTTGGCAGAACTCCAGGGCCTTCAGAACAATGTGG  280

281  AGGACTACGTGAGTGCATTGTCCAGCTGGCAGAAGAACCC  320

321  TGTTAGCTCCAGAAATCCTCACAGCCAAGGTAGGATCAGA  360

361  GAGTTGTTCTCTCAAGCCGAATCCCACTTCAGAAATTCCA  400
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```
401   TGCCTAGCTTTGCTATCTCCGGTTACGAGGTTCTTTTCCT   440
441   CACTACCTATGCTCAAGCTGCCAACACCCACTTGTTTCTC   480
481   CTTAAGGACGCTCAAATCTATGGAGAAGAGTGGGGATACG   520
521   AGAAAGAGGACATTGCTGAGTTCTACAAGCGTCAACTTAA   560
561   GCTCACCCAAGAGTACACTGACCATTGCGTGAAATGGTAT   600
601   AACGTTGGTCTCGATAAGCTCAGAGGCTCTTCCTACGAGT   640
641   CTTGGGTGAACTTCAACAGATACAGGAGAGAGATGACCTT   680
681   GACTGTGCTCGATCTTATCGCACTCTTTCCCTTGTACGAT   720
721   GTGAGACTCTACCCAAAGGAAGTGAAAACTGAGCTTACCA   760
761   GAGACGTGCTCACTGACCCTATTGTCGGAGTCAACAACCT   800
801   TAGGGGTTATGGAACTACCTTCAGCAATATCGAAAACTAC   840
841   ATTAGGAAACCACATCTCTTCGACTATCTTCACAGAATTC   880
881   AATTCCACACAAGGTTTCAACCAGGATACTATGGTAACGA   920
921   CTCCTTCAACTATTGGTCCGGTAACTATGTTTCCACCAGA   960
961   CCAAGCATTGGATCTAATGACATCATCACATCTCCCTTCT   1000
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1001  ATGGTAACAAGTCCAGTGAACCTGTGCAGAACCTTGAGTT  1040
1041  CAACGGCGAGAAAGTCTATAGAGCCGTCGCAAACACCAAT  1080
1081  CTCGCTGTGTGGCCATCCGCAGTTTACTCAGGCGTCACAA  1120
1121  AGGTGGAGTTTAGTCAGTATAACGATCAGACCGATGAGGC  1160
1161  CAGCACCCAGACTTACGACTCCAAACGTAACGTTGGCGCA  1200
1201  GTCTCTTGGGATTCTATCGACCAATTGCCTCCAGAAACCA  1240
1241  CAGACGAACCATTGGAGAAGGGCTACAGCCACCAACTTAA  1280
1281  CTATGTGATGTGCTTCTTGATGCAAGGTTCCAGAGGGACC  1320
1321  ATTCCAGTGTTGACCTGGACACACAAGTCCGTGGACTTCT  1360
1361  TCAACATGATCGATAGCAAGAAGATCACTCAACTTCCCTT  1400
1401  GGTGAAAGCCTACAAGCTGCAATCTGGTGCTTCCGTTGTC  1440
1441  GCAGGTCCCAGATTCACTGGAGGTGACATCATCCAGTGCA  1480
1481  CAGAGAACGGCAGCGCAGCTACTATCTACGTGACACCTGA  1520
1521  TGTGTCTTACTCTCAGAAGTACAGGGCACGTATTCATTAC  1560
1561  GCATCTACCAGCCAGATCACCTTCACACTCAGCTTGGATG  1600
```

1601 GAGCACCCTTCAACCAGTATTACTTTGACAAGACCATCAA 1640

1641 CAAAGGTGACACTCTCACATACAATAGCTTCAACTTGGCA 1680

1681 AGTTTCAGCACACCATTTGAACTCTCAGGCAACAATCTTC 1720

1721 AGATCGGCGTCACCGGTCTCAGCGCCGGAGACAAAGTCTA 1760

1761 CATCGACAAGATTGAGTTCATCCCAGTGAAC 1791.

I. A structural gene which encodes an insecticidal protein of *B.t.* entomocidus having the sequence:

1 ATGGAGGAGAACAACCAAAACCAATGCATTCCATACAACT 40

41 GCTTGAGTAACCCAGAAGAGGTATTGCTTGATGGAGAACG 80

81 CATTTCAACCGGTAACTCTTCCATCGACATCTCCTTGTCC 120

121 TTGGTCCAGTTTCTGGTCAGCAACTTCGTGCCAGGTGGTG 160

161 GGTTCCTTGTCGGACTAATTGACTTCGTCTGGGGTATCGT 200

201 TGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATTGAG 240

241 CAGTTGATCAACGAGAGGATCGCTGAGTTCGCCAGGAACG 280

281 CTGCCATCGCTAACTTGGAAGGATTGGGCAATAACTTCAA 320

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321  CATCTATGTGGAGGCCTTCAAAGAGTGGGAAGAGGACCCT  360
361  AACAACCCAGAGACCCGCACTAGGGTGATCGACAGATTCA  400
401  GAATCTTGGACGGCCTCTTGGAGAGAGATATCCCATCCTT  440
441  CAGAATCTCTGGCTTCGAAGTTCCTCTCTTGTCCGTGTAC  480
481  GCTCAAGCAGCTAATCTTCACCTCGCTATCCTTCGAGACA  520
521  GTGTCATCTTTGGGGAAAGGTGGGGATTGACCACTATCAA  560
561  CGTCAATGAGAATTACAACAGACTTATCAGGCACATTGAC  600
601  GAGTACGCCGACCACTGTGCTAACACCTACAACCGTGGCT  640
641  TGAACAATCTCCCTAAGTCTACTTATCAAGATTGGATTAC  680
681  CTACAACAGGTTGAGGAGAGACTTGACCCTCACAGTTTTG  720
721  GACATTGCAGCTTTCTTCCCGAACTATGACAACAGGAGAT  760
761  ACCCTATCCAACCAGTGGGTCAACTTACCAGAGAAGTCTA  800
801  TACTGACCCACTTATCAACTTCAACCCTCAGTTGCAAAGT  840
841  GTCGCCCAACTTCCCACATTCAACGTCATGGAGTCCAGCC  880
881  GTATCAGGAACCCACACTTGTTTGACATCTTGAACAACCT  920
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 921  TACTATCTTCACCGATTGGTTCAGCGTTGGGCGTAACTTC   960
 961  TATTGGGGTGGACACAGGGTCATCTCCTCTCTTATTGGAG  1000
1001  GTGGGAACATTACCTCTCCTATCTATGGACGTGAGGCAAA  1040
1041  CCAGGAGCCACCACGTAGTTTCACCTTCAACGGTCCAGTC  1080
1081  TTCAGAACCTTGTCTAACCCTACCTTGAGATTGCTCCAGC  1120
1121  AACCTTGGCCAGCTCCACCTTTCAACCTTAGAGGTGTTGA  1160
1161  GGGCGTTGAGTTCTCTACTCCTACCAACTCCTTCACTTAC  1200
1201  AGAGGTAGAGGAACCGTTGATTCCTTGACCGAACTCCCAC  1240
1241  CAGAGGACAATAGCGTGCCACCCAGGGAAGGCTACTCCCA  1280
1281  CAGGTTGTGCCACGCAACCTTCGTGCAGCGTTCCGGAACT  1320
1321  CCATTCCTCACTACAGGAGTTGTGTTCTCATGGACTGATC  1360
1361  GTAGTGCTACTCTCACTAATACCATTGATCCCGAGAGGAT  1400
1401  CAATCAAATCCCATTGGTCAAGGGTTTCCGTGTGTGGGGA  1440
1441  GGAACTTCTGTCATCACAGGACCAGGCTTCACAGGAGGTG  1480
1481  ATATTCTTAGAAGAAACACTTTTGGCGACTTTGTGAGCCT  1520
```

1521 CCAAGTTAACATCAACTCTCCAATTACTCAAAGATATCGT 1560

1561 CTCAGGTTTCGTTACGCATCTTCCCGTGACGCTAGAGTCA 1600

1601 TCGTGCTCACCGGAGCAGCTTCTACCGGTGTCGGTGGACA 1640

1641 AGTCTCCGTGAACATGCCACTCCAGAAGACTATGGAGATC 1680

1681 GGCGAGAACTTGACATCCAGGACCTTCAGATACACCGACT 1720

1721 TCTCTAACCCTTTCAGTTTCCGTGCCAACCCTGACATCAT 1760

1761 TGGCATTAGCGAACAACCTCTCTTTGGAGCTGGTAGCATC 1800

1801 TCATCTGGCGAATTGTACATTGACAAGATTGAGATCATTC 1840

1841 TTGCCGACGCTACCTTCGAGGCTGAGTCTGACCTTGAGAG 1880

1881 AGCCCAGAAGGCTGTGAACGCCCTCTTTACCTCCTCTAAT 1920

1921 CAGATTGGCTTGAAAACTGACGTTACTGACTATCACATTG 1960

1961 ACCAAGTGTCCAACTTGGTCGACTGCCTTAGCGATGAGTT 2000

2001 CTGCCTCGACGAGAAGCGTGAACTCTCCGAGAAAGTTAAA 2040

2041 CACGCCAAGCGTCTCAGCGACGAGAGGAATCTCTTGCAAG 2080

2081 ACCCCAACTTCAGAGGCATCAACAGGCAGCCAGACCGTGG 2120

2121 TTGGAGAGGAAGCACCGACATCACCATCCAAGGAGGCGAC 2160

2161 GATGTGTTCAAGGAGAACTACGTCACCCTCCCAGGAACTG 2200

2201 TGGACGAGTGCTACCCTACCTACTTGTACCAGAAGATCGA 2240

2241 TGAGTCCAAACTCAAAGCCTACACCAGGTATGAACTTAGA 2280

2281 GGCTACATCGAAGACAGCCAAGACCTTGAAATCTACCTCA 2320

2321 TCAGGTACAATGCCAAGCACGAGATCGTGAATGTCCCAGG 2360

2361 TACTGGTTCCCTCTGGCCACTTTCTGCCCAAATGCCCATT 2400

2401 GGGAAGTGTGGAGAGCCTAACAGATGCGCTCCACACCTTG 2440

2441 AGTGGAATCCTGACTTGGACTGCTCCTGCAGGGATGGCGA 2480

2481 GAAGTGTGCCCACCATTCTCATCACTTCACCTTGGACATC 2520

2521 GATGTGGGATGTACTGACCTGAATGAGGACCTCGGAGTCT 2560

2561 GGGTCATCTTCAAGATCAAGACCCAAGACGGACACGCAAG 2600

2601 ACTTGGCAACCTTGAGTTTCTCGAAGAGAAACCATTGCTC 2640

2641 GGTGAAGCTCTCGCTCGTGTGAAGAGAGCAGAGAAGAAGT 2680

2681 GGAGGGACAAACGTGAGAAACTCCAACTCGAGACTAACAT 2720

2721 CGTTTACAAGGAGGCCAAAGAGTCCGTGGATGCTTTGTTC 2760

2761 GTGAACTCCCAATATGATAGGTTGCAAGTGGACACCAACA 2800

2801 TCGCCATGATCCACGCTGCAGACAAACGTGTGCACAGGAT 2840

2841 TCGTGAGGCTTACTTGCCTGAGTTGTCCGTGATCCCTGGT 2880

2881 GTGAACGCTGCCATCTTCGAGGAACTTGAGGGACGTATCT 2920

2921 TTACCGCATACTCCTTGTACGATGCCAGAAACGTCATCAA 2960

2961 GAACGGTGACTTCAACAATGGCCTCTTGTGCTGGAATGTG 3000

3001 AAAGGTCATGTGGACGTGGAGGAACAGAACAATCACCGTT 3040

3041 CCGTCCTGGTTATCCCTGAGTGGGAAGCTGAAGTGTCCCA 3080

3081 AGAGGTTAGAGTCTGTCCAGGTAGAGGCTACATTCTCCGT 3120

3121 GTGACCGCTTACAAGGAGGGATACGGTGAGGGTTGCGTGA 3160

3161 CCATCCACGAGATCGAGGACAACACCGACGAGCTTAAGTT 3200

3201 CTCCAACTGCGTCGAGGAAGAAGTCTATCCCAACAACACC 3240

3241 GTTACTTGCAACAACTACACTGGGACCCAGGAAGAGTACG 3280

3281 AAGGTACCTACACTAGCCGTAACCAAGGTTACGACGAAGC 3320

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3321  TTACGGAAACAATCCTTCCGTTCCTGCTGACTATGCCTCC  3360
3361  GTGTACGAGGAGAAATCCTACACAGATGGCAGACGTGAGA  3400
3401  ACCCTTGCGAGTCCAACAGAGGTTACGGTGACTACACACC  3440
3441  ACTTCCAGCAGGCTATGTTACCAAGGACCTTGAGTACTTT  3480
3481  CCTGAGACCGACAAAGTGTGGATCGAGATCGGTGAAACCG  3520
3521  AGGGAACCTTCATCGTGGACAGCGTGGAGCTTCTCTTGAT  3560
3561  GGAGGAA  3567.
```

J. A structural gene which encodes a P2 insecticidal protein having the sequence:

```
  1  ATGGACAACAACGTCTTGAACTCTGGTAGAACAACCATCT  40
 41  GCGACGCATACAACGTCGTGGCTCACGATCCATTCAGCTT  80
 81  CGAACACAAGAGCCTCGACACTATTCAGAAGGAGTGGATG  120
121  GAATGGAAACGTACTGACCACTCTCTCTACGTCGCACCTG  160
161  TGGTTGGAACAGTGTCCAGCTTCCTTCTCAAGAAGGTCGG  200
201  CTCTCTCATCGGAAAACGTATCTTGTCCGAACTCTGGGGT  240
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241  ATCATCTTTCCATCTGGGTCCACTAATCTCATGCAAGACA  280
281  TCTTGAGGGAGACCGAACAGTTTCTCAACCAGCGTCTCAA  320
321  CACTGATACCTTGGCTAGAGTCAACGCTGAGTTGATCGGT  360
361  CTCCAAGCAAACATTCGTGAGTTCAACCAGCAAGTGGACA  400
401  ACTTCTTGAATCCAACTCAGAATCCTGTGCCTCTTTCCAT  440
441  CACTTCTTCCGTGAACACTATGCAGCAACTCTTCCTCAAC  480
481  AGATTGCCTCAGTTTCAGATTCAAGGCTACCAGTTGCTCC  520
521  TTCTTCCACTCTTTGCTCAGGCTGCCAACATGCACTTGTC  560
561  CTTCATACGTGACGTGATCCTCAACGCTGACGAATGGGGA  600
601  ATCTCTGCAGCCACTCTTAGGACATACAGAGACTACTTGA  640
641  GGAACTACACTCGTGATTACTCCAACTATTGCATCAACAC  680
681  TTATCAGACTGCCTTTCGTGGACTCAATACTAGGCTTCAC  720
721  GACATGCTTGAGTTCAGGACCTACATGTTCCTTAACGTGT  760
761  TTGAGTACGTCAGCATTTGGAGTCTCTTCAAGTACCAGAG  800
801  CTTGATGGTGTCCTCTGGAGCCAATCTCTACGCCTCTGGC  840
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```
841   AGTGGACCACAGCAAACTCAGAGCTTCACAGCTCAGAACT   880
881   GGCCATTCTTGTATAGCTTGTTCCAAGTCAACTCCAACTA   920
921   CATTCTCAGTGGTATCTCTGGGACCAGACTCTCCATAACC   960
961   TTTCCCAACATTGGTGGACTTCCAGGCTCCACTACAACCC   1000
1001  ATAGCCTTAACTCTGCCAGAGTGAACTACAGTGGAGGTGT   1040
1041  CAGCTCTGGATTGATTGGTGCAACTAACTTGAACCACAAC   1080
1081  TTCAATTGCTCCACCGTCTTGCCACCTCTGAGCACACCGT   1120
1121  TTGTGAGGTCCTGGCTTGACAGCGGTACTGATCGCGAAGG   1160
1161  AGTTGCTACCTCTACAAACTGGCAAACCGAGTCCTTCCAA   1200
1201  ACCACTCTTAGCCTTCGGTGTGGAGCTTTCTCTGCACGTG   1240
1241  GGAATTCAAACTACTTTCCAGACTACTTCATTAGGAACAT   1280
1281  CTCTGGTGTTCCTCTCGTCATCAGGAATGAAGACCTCACC   1320
1321  CGTCCACTTCATTACAACCAGATTAGGAACATCGAGTCTC   1360
1361  CATCCGGTACTCCAGGAGGTGCAAGAGCTTACCTCGTGTC   1400
1401  TGTCCATAACAGGAAGAACAACATCTACGCTGCCAACGAG   1440
```

```
1441  AATGGCACCATGATTCACCTTGCACCAGAAGATTACACTG  1480

1481  GATTCACCATCTCTCCAATCCATGCTACCCAAGTGAACAA  1520

1521  TCAGACACGCACCTTCATCTCCGAAAAGTTCGGAAATCAA  1560

1561  GGTGACTCCTTGAGGTTCGAGCAATCCAACACTACCGCTA  1600

1601  GGTACACTTTGAGAGGCAATGGAAACAGCTACAACCTTTA  1640

1641  CTTGAGAGTTAGCTCCATTGGTAACTCCACCATCCGTGTT  1680

1681  ACCATCAACGGACGTGTTTACACAGTCTCTAATGTGAACA  1720

1721  CTACAACGAACAATGATGGCGTTAACGACAACGGAGCCAG  1760

1761  ATTCAGCGACATCAACATTGGCAACATCGTGGCCTCTGAC  1800

1801  AACACTAACGTTACTTTGGACATCAATGTGACCCTCAATT  1840

1841  CTGGAACTCCATTTGATCTCATGAACATCATGTTTGTGCC  1880

1881  AACTAACCTCCCTCCATTGTAC  1902;      or
```

K. A structural gene sequence encoding a. fusion protein comprising the N-terminal 610 amino acids of *B.t.k.* HD-1 and the C-terminal 567 amino acids of *B.t.k.* HD-73, said gene having the sequence:

```
  1  ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA   40
 41  ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA   80
 81  ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG  120
121  TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG  160
161  CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTAT  200
201  CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT  240
241  GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA  280
281  ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA  320
321  CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT  360
361  CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT  400
401  TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT  440
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```
 441  GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG   480

 481  TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG   520

 521  ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC   560

 561  AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT   600

 601  GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG   640

 641  GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT   680

 681  TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT   720

 721  TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA   760

 761  CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT   800

 801  CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC   840

 841  CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC   880

 881  CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC   920

 921  CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG   960

 961  ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA  1000

1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA  1040
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```
1041  ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA  1080
1081  ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG  1120
1121  GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA  1160
1161  GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT  1200
1201  TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC  1240
1241  CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC  1280
1281  CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC  1320
1321  AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT  1360
1361  CATGGATTCATCGTAGTGCTGAGTTCAACAATATCATTCC  1400
1401  TTCCTCTCAAATCACCCAAATCCCATTGACCAAGTCTACT  1440
1441  AACCTTGGATCTGGAACTTCTGTCGTGAAAGGACCAGGCT  1480
1481  TCACAGGAGGTGATATTCTTAGAAGAACTTCTCCTGGCCA  1520
1521  GATTAGCACCCTCAGAGTTAACATCACTGCACCACTTTCT  1560
1561  CAAAGATATCGTGTCAGGATTCGTTACGCATCTACCACTA  1600
1601  ACTTGCAATTCCACACCTCCATCGACGGAAGGCCTATCAA  1640
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1641  TCAGGGTAACTTCTCCGCAACCATGTCAAGCGGCAGCAAC  1680
1681  TTGCAATCCGGCAGCTTCAGAACCGTCGGTTTCACTACTC  1720
1721  CTTTCAACTTCTCTAACGGATCAAGCGTTTTCACCCTTAG  1760
1761  CGCTCATGTGTTCAATTCTGGCAATGAAGTGTACATTGAC  1800
1801  CGTATTGAGTTTGTGCCTGCCGAAGTTACCCTCGAGGCTG  1840
1841  AGTACAACCTTGAGAGAGCCCAGAAGGCTGTGAACGCCCT  1880
1881  CTTTACCTCCACCAATCAGCTTGGCTTGAAAACTAACGTT  1920
1921  ACTGACTATCACATTGACCAAGTGTCCAACTTGGTCACCT  1960
1961  ACCTTAGCGATGAGTTCTGCCTCGACGAGAAGCGTGAACT  2000
2001  CTCCGAGAAAGTTAAACACGCCAAGCGTCTCAGCGACGAG  2040
2041  AGGAATCTCTTGCAAGACTCCAACTTCAAAGACATCAACA  2080
2081  GGCAGCCAGAACGTGGTTGGGGTGGAAGCACCGGGATCAC  2120
2121  CATCCAAGGAGGCGACGATGTGTTCAAGGAGAACTACGTC  2160
2161  ACCCTCTCCGGAACTTTCGACGAGTGCTACCCTACCTACT  2200
2201  TGTACCAGAAGATCGATGAGTCCAAACTCAAAGCCTTCAC  2240
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2241  CAGGTATCAACTTAGAGGCTACATCGAAGACAGCCAAGAC  2280
2281  CTTGAAATCTACTCGATCAGGTACAATGCCAAGCACGAGA  2320
2321  CCGTGAATGTCCCAGGTACTGGTTCCCTCTGGCCACTTTC  2360
2361  TGCCCAATCTCCCATTGGGAAGTGTGGAGAGCCTAACAGA  2400
2401  TGCGCTCCACACCTTGAGTGGAATCCTGACTTGGACTGCT  2440
2441  CCTGCAGGGATGGCGAGAAGTGTGCCCACCATTCTCATCA  2480
2481  CTTCTCCTTGGACATCGATGTGGGATGTACTGACCTGAAT  2520
2521  GAGGACCTCGGAGTCTGGGTCATCTTCAAGATCAAGACCC  2560
2561  AAGACGGACACGCAAGACTTGGCAACCTTGAGTTTCTCGA  2600
2601  AGAGAAACCATTGGTCGGTGAAGCTCTCGCTCGTGTGAAG  2640
2641  AGAGCAGAGAAGAAGTGGAGGGACAAACGTGAGAAACTCG  2680
2681  AATGGGAAACTAACATCGTTTACAAGGAGGCCAAAGAGTC  2720
2721  CGTGGATGCTTTGTTCGTGAACTCCCAATATGATCAGTTG  2760
2761  CAAGCCGACACCAACATCGCCATGATCCACGCCGCAGACA  2800
2801  AACGTGTGCACAGCATTCGTGAGGCTTACTTGCCTGAGTT  2840
```

2841 GTCCGTGATCCCTGGTGTGAACGCTGCCATCTTCGAGGAA 2880

2881 CTTGAGGGACGTATCTTTACCGCATTCTCCTTGTACGATG 2920

2921 CCAGAAACGTCATCAAGAACGGTGACTTCAACAATGGCCT 2960

2961 CAGCTGCTGGAATGTGAAAGGTCATGTGGACGTGGAGGAA 3000

3001 CAGAACAATCAGCGTTCCGTCCTGGTTGTGCCTGAGTGGG 3040

3041 AAGCTGAAGTGTCCCAAGAGGTTAGAGTCTGTCCAGGTAG 3080

3081 AGGCTACATTCTCCGTGTGACCGCTTACAAGGAGGGATAC 3120

3121 GGTGAGGGTTGCGTGACCATCCACGAGATCGAGAACAACA 3160

3161 CCGACGAGCTTAAGTTCTCCAACTGCGTCGAGGAAGAAAT 3200

3201 CTATCCCAACAACACCGTTACTTGCAACGACTACACTGTG 3240

3241 AATCAGGAAGAGTACGGAGGTGCCTACACTAGCCGTAACA 3280

3281 GAGGTTACAACGAAGCTCCTTCCGTTCCTGCTGACTATGC 3320

3321 CTCCGTGTACGAGGAGAAATCCTACACAGATGGCAGACGT 3360

3361 GAGAACCCTTGCGAGTTCAACAGAGGTTACAGGGACTACA 3400

3401 CACCACTTCCAGTTGGCTATGTTACCAAGGAGCTTGAGTA 3440

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3441  CTTTCCTGAGACCGACAAAGTGTGGATCGAGATCGGTGAA  3480

3481  ACCGAGGGAACCTTCATCGTGGACAGCGTGGAGCTTCTCT  3520

3521  TGATGGAGGAA  3531.
```

Patentansprüche

1. Verfahren zur Modifizierung einer Wildtyp-Struktur-Gensequenz, welche für ein insektizides Protein von *Bacillus thuringiensis* codiert, zur Verbesserung der Expression dieses Proteins in Pflanzen, welches umfasst:

    a) das Identifizieren von Regionen innerhalb dieser Sequenz mit mehr als vier aufeinander folgenden Adenin- oder Thymin-Nukleotiden;

    b) das Modifizieren der Regionen von Schritt (a), die zwei oder mehr Polyadenylierungssignale innerhalb einer Zehn-Basen-Sequenz aufweisen, um diese Signale zu entfernen, wobei eine Gensequenz, die für dieses Protein codiert, beibehalten wird; und

    c) das Modifizieren der 15-30-Basen-Regionen, die die Regionen von Schritt (a) umgeben, um Pflanzen-Polyadenylierungs-Hauptsignale, aufeinander folgende Sequenzen, die mehr als ein untergeordnetes Polyadenylierungssignal enthalten, und aufeinander folgende Sequenzen, die mehr als eine ATTTA-Sequenz enthalten, zu entfernen, wobei eine Gensequenz, die für dieses Protein codiert, beibehalten wird.

2. Verfahren zur Modifizierung einer Wildtyp-Struktur-Gensequenz, welche für ein insektizides Protein von *Bacillus thuringiensis* codiert, zur Verbesserung der Expression dieses Proteins in Pflanzen, welches umfasst:

    a) das Entfernen von Polyadenylierungssignalen, die in diesem Wildtyp-Gen enthalten sind, wobei eine Sequenz, die für dieses Protein codiert, beibehalten wird; und

    b) das Entfernen von ATTTA-Sequenzen, die in diesem Wildtyp-Gen enthalten sind, wobei eine Sequenz, die für dieses Protein codiert, beibehalten wird.

3. Verfahren nach Anspruch 2, welches weiters das Entfernen von selbstkomplementären Sequenzen und das Ersetzen solcher Sequenzen durch nicht-selbstkomplementäre DNA, welche von Pflanzen bevorzugte Codons aufweist, wobei eine Struktur-Gensequenz, die für dieses Protein codiert, beibehalten wird.

4. Verfahren nach den Ansprüchen 1 bis 3, welches weiters die Verwendung von von Pflanzen bevorzugten Sequenzen beim Entfernen der Polyadenylierungssignale und ATTTA-Sequenzen umfasst.

5. Verfahren nach den Ansprüchen 1 bis 3, bei welchem die Pflanzen-Polyadenylierungssignale ausgewählt sind aus der Gruppe bestehend aus AATAAA, AATAAT, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA und CATAAA.

6. Verfahren zur Verbesserung der Expression eines heterologen Gens in Pflanzen, wobei dieses Gen ein modifiziertes chimäres Gen aufweist, das einen Promotor enthält, der in Pflanzenzellen wirkt, der operabel mit einer strukturellen Codiersequenz und einer 3'-nicht-translatierten Region, die ein Polyadenylierungssignal enthält, das in Pflanzen wirkt, um die Addition von Polyadenylat-Nukleotiden an das 3'-Ende der RNA zu bewirken, verbunden ist, wobei die strukturelle Codiersequenz für ein insektizides Protein codiert, von welchem mindestens ein Teil von einem *Bacillus-thuringiensis*-Protein stammte, wobei das Verfahren das Modifizieren dieser strukturellen Codier-

sequenz umfasst, so dass diese Sequenz eine DNA-Sequenz aufweist, die sich von der natürlicherweise vorkommenden DNA-Sequenz, welche für dieses *Bacillus-thuringiensis*-Protein codiert, unterscheidet und diese strukturelle Codiersequenz nicht mehr als 5 aufeinander folgende Nukleotide aufweist, die entweder aus Adenin- oder aus Thymin-Resten bestehen.

7. Verfahren zur Verbesserung der Expression eines heterologen Gens in Pflanzen, wobei dieses Gen ein modifiziertes chimäres Gen aufweist, das einen Promotor enthält, der in Pflanzenzellen wirkt, der operabel mit einer strukturellen Codiersequenz und einer 3'-nicht-translatierten Region, die ein Polyadenylierungssignal enthält, das in Pflanzen wirkt, um die Addition von Polyadenylat-Nukleotiden an das 3'-Ende der RNA zu bewirken, verbunden ist, wobei diese strukturelle Codiersequenz für ein insektizides Protein codiert, von welchem mindestens ein Teil von einem *Bacillus-thuringiensis*-Protein stammte, wobei das Verfahren das Modifizieren dieser strukturellen Codiersequenz umfasst, so dass diese Sequenz eine DNA-Sequenz besitzt, die sich von der natürlicherweise vorkommenden DNA-Sequenz, die für das *Bacillus-thuringiensis*-Protein codiert, unterscheidet und die folgenden Merkmale hat:

diese strukturelle Codiersequenz hat eine Region, die zur folgenden Sequenz komplementär ist:

```
GGCTTGATTCCTAGCGAACTCTTCGATTCTCTGGTTGATGAGCTGTTC
1   5    10   15   20   25   30   35   40   45
```

wobei in der Codiersequenz dieser Region 2 AACCAA- und 1 AATTAA-Sequenz eliminiert sind.

8. Verfahren nach Anspruch 7, wobei die strukturelle Codiersequenz für ein insektizides Protein codiert, von welchem mindestens ein Teil von einem *Bacillus thuringiensis kurstakis* HD-1 stammte.

9. Verfahren nach Anspruch 7 oder 8, wobei die Pflanze eine Tabakpflanze ist.

10. Modifiziertes chimäres Gen, das einen Promotor enthält, welcher in Pflanzenzellen wirkt, der operabel mit einer strukturellen Codiersequenz und einer 3'-nicht-translatierten Region, die ein Polyadenylierungssignal enthält, welches in Pflanzen wirkt, um die Addition von Polyadenylat-Nukleotiden am 3'-Ende der RNA zu bewirken, verbunden ist, wobei diese strukturelle Codiersequenz für ein insektizides Protein codiert, von welchem mindestens ein Teil von einem *Bacillus thuringiensis*-Protein stammt, wobei diese strukturelle Codiersequenz eine DNA-Sequenz aufweist, die sich von der natürlicherweise vorkommenden DNA-Sequenz, welche für dieses *Bacillus thuringiensis*-Protein codiert, unterscheidet und ausgewählt ist aus:

A. einem Struktur-Gen, welches für ein insektizides Protein von *B.t.k.* HD-1 codiert, mit der Sequenz:

```
  1 ATGGCTATAGAAACTGGTTACACCCCAATCGATATTTCCT  40
 41 TGTCGCTAACGCAATTTCTTTTGAGTGAATTTGTTCCCGG  80
 81 TGCTGGATTTGTGTTAGGACTAGTTGATATTATCTGGGGA 120
121 ATTTTTGGTCCCTCTCAATGGGACGCATTTCTTGTACAAA 160
161 TTGAACAGCTCATCAACCAGAGAATCGAAGAGTTCGCTAG 200
201 GAATCAAGCCATTTCTAGATTAGAAGGACTAAGCAATCTT 240
241 TATCAAATTTACGCAGAATCTTTTAGAGAGTGGGAAGCAG 280
281 ATCCTACTAATCCAGCATTAAGAGAAGAGATGCGTATTCA 320
321 ATTCAATGACATGAACAGTGCCCTTACAACCGCTATTCCT 360
361 CTTTTTGCAGTTCAAAATTATCAAGTTCCTCTCCTCTCCG 400
401 TGTACGTTCAAGCTGCCAACCTCCACCTCTCAGTTTTGAG 440
441 AGATGTTTCAGTGTTTGGACAAAGGTGGGGATTTGATGCC 480
481 GCGACTATCAATAGTCGTTATAATGATTTAACTAGGCTTA 520
521 TTGGCAACTATACAGATCATGCTGTACGCTGGTACAATAC 560
561 GGGATTAGAGCGTGTATGGGGACCGGATTCTAGAGATTGG 600
601 ATCAGGTACAACCAGTTCAGAAGAGAGCTTACACTAACTG 640
641 TATTAGATATCGTTTCTCTATTTCCGAACTATGATAGTAG 680
681 AACGTATCCAATTCGAACAGTTTCCCAATTAACAAGAGAA 720
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 721  ATTTATACAAACCCAGTATTAGAAAATTTTGATGGTAGTT   760
 761  TTCGAGGCTCGGCTCAGGGCATAGAAGGAAGTATTAGGAG   800
 801  TCCACATTTGATGGATATACTTAATAGTATAACCATCTAT   840
 841  ACGGATGCTCATAGAGGAGAATACTACTGGTCCGGTCACC   880
 881  AGATCATGGCTTCTCCTGTAGGGTTTTCGGGGCCAGAATT   920
 921  CACTTTTCCGCTATATGGAACTATGGGAAATGCAGCTCCA   960
 961  CAACAACGTATTGTTGCTCAACTAGGTCAGGGCGTGTATA  1000
1001  GAACATTATCGTCCACCTTATATAGAAGACCTTTTAACAT  1040
1041  CGGGATCAACAACCAACAACTATCTGTTCTTGACGGGACA  1080
1081  GAATTTGCTTATGGAACCTCCTCAAATTTGCCATCCGCTG  1120
1121  TATACAGAAAAAGCGGAACGGTAGATTCGCTGGATGAAAT  1160
1161  ACCGCCACAGAATAACAACGTGCCACCTAGGCAAGGATTT  1200
1201  AGTCATCGATTAAGCCATGTTTCAATGTTTCGTTCAGGCT  1240
1241  TTAGTAATAGTAGTGTAAGTATAATAAGAGCTCCTATGTT  1280
1281  CTCTTGGATACATCGTAGTGCTGAGTTCAACAACATCATC  1320
1321  CCTTCATCACAAATCACCCAAATCCCACTCACCAAGTCTA  1360
1361  CTAATCTTGGCTCTGGAACTTCTGTCGTTAAAGGACCAGG  1400
1401  ATTTACAGGAGGAGATATTCTTCGAAGAACTTCACCTGGC  1440
```

1441 CAGATTTCAACCTTAAGAGTAAATATTACTGCACCATTAT 1480

1481 CACAAAGATATCGGGTAAGAATTCGCTACGCTTCTACCAC 1520

1521 AAACCTTCAGTTCCACACATCAATTGACGGAAGACCTATT 1560

1561 AATCAGGGGAATTTTTCAGCAACTATGAGTAGTGGGAGTA 1600

1601 ATTTACAGTCCGGAAGCTTTAGGACTGTAGGTTTTACTAC 1640

1641 TCCGTTTAACTTTTCAAATGGATCAAGTGTATTTACGTTA 1680

1681 AGTGCTCATGTCTTCAATTCAGGCAATGAAGTTTATATAG 1720

1721 ATCGAATTGAATTTGTTCCGGCA 1743.

B. einem Struktur-Gen, welches für ein insektizides Protein von *B.t.k.* HD-73 codiert, mit der Sequenz:

1 ATGGCCATTGAAACCGGTTACACTCCCATCGACATCTCCT 40

41 TGTCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGG 80

81 TGCTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGT 120

121 ATCTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAA 160

161 TTGAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAG 200

201 GAACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTC 240

241 TACCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCG 280

281 ATCCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCA 320

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321   ATTCAACGACATGAACAGCGCCTTGACCACAGCTATCCCA   360
361   TTGTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCG   400
401   TGTACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCG   440
441   AGACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCT   480
481   GCAACCATCAATAGCCGTTACAACGACCTTACTAGGCTGA   520
521   TTGGAAACTACACCGACCACGCTGTTCGTTGGTACAACAC   560
561   TGGCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGG   600
601   ATTAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAG   640
641   TTTTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAG   680
681   AACCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAA   720
721   ATCTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCT   760
761   TCCGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAG   800
801   CCCACACTTGATGGACATCTTGAACAGCATAACTATCTAC   840
841   ACCGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACC   880
881   AGATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTT   920
921   TACCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCA   960
961   CAACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACA   1000
1001  GAACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATAT   1040
1041  CGGTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACA   1080
```

1081 GAGTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTG 1120

1121 TTTACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAAT 1160

1161 CCCACCACAGAACAACAATGTGCCACCCAGGCAAGGATTC 1200

1201 TCCCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGAT 1240

1241 TCAGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTT 1280

1281 CTCTTGGATACACCGTAGTGCTGAGTTCAACAACATCATC 1320

1321 GCATCCGATAGTATTACTCAAATCCCTGCAGTGAAGGGAA 1360

1361 ACTTTCTCTTCAACGGTTCTGTCATTTCAGGACCAGGATT 1400

1401 CACTGGTGGAGACCTCGTTAGACTCAACAGCAGTGGAAAT 1440

1441 AACATTCAGAATAGAGGGTATATTGAAGTTCCAATTCACT 1480

1481 TCCCATCCACATCTACCAGATATAGAGTTCGTGTGAGGTA 1520

1521 TGCTTCTGTGACCCCTATTCACCTCAACGTTAATTGGGGT 1560

1561 AATTCATCCATCTTCTCCAATACAGTTCCAGCTACAGCTA 1600

1601 CCTCCTTGGATAATCTCCAATCCAGCGATTTCGGTTACTT 1640

1641 TGAAAGTGCCAATGCTTTTACATCTTCACTCGGTAACATC 1680

1681 GTGGGTGTTAGAAACTTTAGTGGGACTGCAGGAGTGATTA 1720

1721 TCGACAGATTCGAGTTCATTCCAGTTACTGCAACACTCGA 1760

1761 GGCTGAG 1767.

C. einem Struktur-Gen, das für ein insektizides Protein von *B.t.k.* HD-1 codiert, mit der Sequenz:

```
  1  ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA   40
 41  ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA   80
 81  ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG  120
121  TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG  160
161  CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTAT  200
201  CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT  240
241  GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA  280
281  ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA  320
321  CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT  360
361  CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT  400
401  TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT  440
441  GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG  480
481  TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG  520
521  ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC  560
561  AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT  600
601  GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG  640
641  GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT  680
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```
 681  TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT   720
 721  TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA   760
 761  CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT   800
 801  CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC   840
 841  CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC   880
 881  CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC   920
 921  CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG   960
 961  ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA  1000
1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA  1040
1041  ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA  1080
1081  ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG  1120
1121  GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA  1160
1161  GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT  1200
1201  TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC  1240
1241  CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC  1280
1281  CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC  1320
1321  AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT  1360
1361  CATGGATTCATCGTAGTGCTGAGTTCAACAATATCATTCC  1400
1401  TTCCTCTCAAATCACCCAAATCCCATTGACCAAGTCTACT  1440
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1441  AACCTTGGATCTGGAACTTCTGTCGTGAAAGGACCAGGCT  1480

1481  TCACAGGAGGTGATATTCTTAGAAGAACTTCTCCTGGCCA  1520

1521  GATTAGCACCCTCAGAGTTAACATCACTGCACCACTTTCT  1560

1561  CAAAGATATCGTGTCAGGATTCGTTACGCATCTACCACTA  1600

1601  ACTTGCAATTCCACACCTCCATCGACGGAAGGCCTATCAA  1640

1641  TCAGGGTAACTTCTCCGCAACCATGTCAAGCGGCAGCAAC  1680

1681  TTGCAATCCGGCAGCTTCAGAACCGTCGGTTTCACTACTC  1720

1721  CTTTCAACTTCTCTAACGGATCAAGCGTTTTCACCCTTAG  1760

1761  CGCTCATGTGTTCAATTCTGGCAATGAAGTGTACATTGAC  1800

1801  CGTATTGAGTTTGTGCCTGCCGAAGTTACCTTCGAGGCTG  1840

1841  AGTAC  1845.
```

D. einem Struktur-Gen, das für ein insektizides Protein codiert, das von *B.t.k.* HD-73 stammt, mit der Sequenz:

```
  1  ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA  40

 41  ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA  80

 81  ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG  120

121  TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG  160

161  CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTAT  200
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201  CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT  240
241  GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA  280
281  ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA  320
321  CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT  360
361  CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT  400
401  TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT  440
441  GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG  480
481  TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG  520
521  ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC  560
561  AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT  600
601  GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG  640
641  GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT  680
681  TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT  720
721  TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA  760
761  CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT  800
801  CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC  840
841  CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC  880
881  CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC  920
921  CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG  960
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```
961   ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA   1000
1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA   1040
1041  ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA   1080
1081  ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG   1120
1121  GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA   1160
1161  GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT   1200
1201  TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC   1240
1241  CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC   1280
1281  CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC   1320
1321  AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT   1360
1361  CTTGGATACACCGTAGTGCTGAGTTCAACAACATCATCGC   1400
1401  ATCCGATAGTATTACTCAAATCCCTGCAGTGAAGGGAAAC   1440
1441  TTTCTCTTCAACGGTTCTGTCATTTCAGGACCAGGATTCA   1480
1481  CTGGTGGAGACCTCGTTAGACTCAACAGCAGTGGAAATAA   1520
1521  CATTCAGAATAGAGGGTATATTGAAGTTCCAATTCACTTC   1560
1561  CCATCCACATCTACCAGATATAGAGTTCGTGTGAGGTATG   1600
1601  CTTCTGTGACCCCTATTCACCTCAACGTTAATTGGGGTAA   1640
1641  TTCATCCATCTTCTCCAATACAGTTCCAGCTACAGCTACC   1680
1681  TCCTTGGATAATCTCCAATCCAGCGATTTCGGTTACTTTG   1720
```

1721 AAAGTGCCAATGCTTTTACATCTTCACTCGGTAACATCGT 1760

1761 GGGTGTTAGAAACTTTAGTGGGACTGCAGGAGTGATTATC 1800

1801 GACAGATTCGAGTTCATTCCAGTTACTGCAACACTCGAGG 1840

1841 CTGAATATAATCTGGAAAGAGCGCAGAAGGCGGTAATGCG 1880

1881 CTGTTTACGTCTACAAACCAGCTTGGACTCAAGACAAATG 1920

1921 G 1921

E. einem Struktur-Gen, das für das insektizide Protein von *B.t.k.* HD-73 in dessen gesamter Länge codiert, mit der Sequenz:

1 ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA 40

41 ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA 80

81 ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG 120

121 TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG 160

161 CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTAT 200

201 CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT 240

241 GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA 280

281 ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA 320

321 CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT 360

361 CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT 400

401 TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT 440

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441   GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG   480
481   TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG   520
521   ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC   560
561   AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT   600
601   GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG   640
641   GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT   680
681   TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT   720
721   TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA   760
761   CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT   800
801   CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC   840
841   CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC   880
881   CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC   920
921   CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG   960
961   ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA   1000
1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA   1040
1041  ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA   1080
1081  ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG   1120
1121  GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA   1160
1161  GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT   1200
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1201 TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC 1240

1241 CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC 1280

1281 CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC 1320

1321 AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT 1360

1361 CTTGGATACACCGTAGTGCTGAGTTCAACAACATCATCGC 1400

1401 ATCCGATAGTATTACTCAAATCCCTGCAGTGAAGGGAAAC 1440

1441 TTTCTCTTCAACGGTTCTGTCATTTCAGGACCAGGATTCA 1480

1481 CTGGTGGAGACCTCGTTAGACTCAACAGCAGTGGAAATAA 1520

1521 CATTCAGAATAGAGGGTATATTGAAGTTCCAATTCACTTC 1560

1561 CCATCCACATCTACCAGATATAGAGTTCGTGTGAGGTATG 1600

1601 CTTCTGTGACCCCTATTCACCTCAACGTTAATTGGGGTAA 1640

1641 TTCATCCATCTTCTCCAATACAGTTCCAGCTACAGCTACC 1680

1681 TCCTTGGATAATCTCCAATCCAGCGATTTCGGTTACTTTG 1720

1721 AAAGTGCCAATGCTTTTACATCTTCACTCGGTAACATCGT 1760

1761 GGGTGTTAGAAACTTTAGTGGGACTGCAGGAGTGATTATC 1800

1801 GACAGATTCGAGTTCATTCCAGTTACTGCAACACTCGAGG 1840

1841 CTGAATATAATCTGGAAAGAGCGCAGAAGGCGGTGAATGC 1880

1881 GCTGTTTACGTCTACAAACCAGCTCGGCCTCAAGACCAAT 1920

1921 GTGACGGATTATCATATTGATCAAGTGTCCAACTTGGTGA 1960

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1961  CCTACCTCAGCGATGAGTTCTGTCTGGATGAAAAGCGAGA  2000
2001  ATTGTCCGAGAAAGTCAAACATGCGAAGCGACTCAGTGAT  2040
2041  GAACGCAATTTACTCCAAGATTCAAATTTCAAAGACATTA  2080
2081  ATAGGCAACCAGAACGTGGGTGGGGCGGAAGTACAGGGAT  2120
2121  TACCATCCAGGGAGGTGACGACGTGTTCAAGGAGAACTAC  2160
2161  GTCACACTATCAGGTACCTTTGATGAGTGCTATCCAACAT  2200
2201  ACCTCTACCAGAAGATCGACGAGTCCAAGTTGAAAGCCTT  2240
2241  TACCCGTTATCAATTAAGAGGGTATATCGAAGATAGTCAA  2280
2281  GACCTCGAGATCTACCTCATCCGCTACAATGCAAAACATG  2320
2321  AAACAGTAAATGTGCCAGGTACGGGTTCCTTATGGCCGCT  2360
2361  TTCAGCCCAAAGTCCAATCGGAAAGTGTGGAGAGCCGAAT  2400
2401  CGATGCGCGCCACACCTTGAATGGAATCCTGACTTAGATT  2440
2441  GTTCGTGTAGGGATGGAGAAAAGTGTGCCCATCATTCGCA  2480
2481  TCATTTCTCCTTAGACATTGATGTAGGATGTACAGACTTA  2520
2521  AATGAGGACCTAGGTGTATGGGTGATCTTTAAGATTAAGA  2560
2561  CGCAAGATGGGCACGCAAGACTAGGGAATCTAGAGTTTCT  2600
2601  CGAAGAGAAACCATTAGTAGGAGAAGCGCTAGCTCGTGTG  2640
2641  AAAAGAGCGGAGAAAAAATGGAGAGACAAACGTGAGAAGT  2680
2681  TGGAATGGGAGACCAACATCGTCTACAAAGAGGCAAAAGA  2720
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2721  ATCTGTAGATGCTTTATTTGTAAACTCTCAATATGATCAA  2760
2761  TTACAAGCGGATACGAATATTGCCATGATTCATGCGGCAG  2800
2801  ATAAACGTGTTCATAGCATTCGAGAAGCTTATCTGCCTGA  2840
2841  GCTGTCTGTGATTCCGGGTGTCAATGCGGCTATTTTTGAA  2880
2881  GAATTAGAAGGGCGTATTTTCACTGCATTCTCCCTCTACG  2920
2921  ATGCCAGAAACGTCATCAAGAACGGTGACTTCAACAATGG  2960
2961  CTTATCCTGCTGGAACGTGAAAGGGCATGTAGATGTAGAA  3000
3001  GAACAAAACAACCAACGTTCGGTCCTTGTTGTTCCGGAAT  3040
3041  GGGAAGCAGAAGTGTCACAAGAAGTTCGTGTCTGTCCGGG  3080
3081  TCGTGGCTATATCCTTCGTGTCACAGCGTACAAGGAGGGA  3120
3121  TATGGAGAAGGTTGCGTAACCATTCATGAGATCGAGAACA  3160
3161  ATACAGACGAACTGAAGTTTAGCAACTGCGTAGAAGAGGA  3200
3201  AATCTATCCAAATAACACGGTAACGTGTAATGATTATACT  3240
3241  GTAAATCAAGAAGAATACGGAGGTGCGTACACTTCTCGTA  3280
3281  ATCGAGGATATAACGAAGCTCCTTCCGTACCAGCTGATTA  3320
3321  TGCGTCAGTCTATGAAGAAAAATCGTATACAGATGGACGA  3360
3361  AGAGAGAATCCTTGTGAATTTAACAGAGGGTATAGGGATT  3400
3401  ACACGCCACTACCAGTTGGTTATGTGACAAAAGAATTAGA  3440
3441  ATACTTCCCAGAAACCGATAAGGTATGGATTGAGATTGGA  3480
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3481 GAAACGGAAGGAACATTTATCGTGGACAGCGTGGAATTAC 3520

3521 TCCTTATGGAGGAA 3534.

F. einem Struktur-Gen, das für ein insektizides Protein von *B.t.k.* HD-73 in dessen gesamter Länge codiert, mit der Sequenz:

1 ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA 40

41 ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA 80

81 ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG 120

121 TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG 160

161 CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTAT 200

201 CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT 240

241 GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA 280

281 ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA 320

321 CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT 360

361 CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT 400

401 TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT 440

441 GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG 480

481 TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG 520

521 ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC 560

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561   AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT   600
601   GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG   640
641   GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT   680
681   TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT   720
721   TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA   760
761   CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT   800
801   CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC   840
841   CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC   880
881   CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC   920
921   CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG   960
961   ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA   1000
1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA   1040
1041  ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA   1080
1081  ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG   1120
1121  GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA   1160
1161  GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT   1200
1201  TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC   1240
1241  CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC   1280
1281  CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC   1320
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1321 AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT 1360

1361 CTTGGATACACCGTAGTGCTGAGTTCAACAACATCATCGC 1400

1401 ATCCGATAGTATTACTCAAATCCCTGCAGTGAAGGGAAAC 1440

1441 TTTCTCTTCAACGGTTCTGTCATTTCAGGACCAGGATTCA 1480

1481 CTGGTGGAGACCTCGTTAGACTCAACAGCAGTGGAAATAA 1520

1521 CATTCAGAATAGAGGGTATATTGAAGTTCCAATTCACTTC 1560

1561 CCATCCACATCTACCAGATATAGAGTTCGTGTGAGGTATG 1600

1601 CTTCTGTGACCCCTATTCACCTCAACGTTAATTGGGGTAA 1640

1641 TTCATCCATCTTCTCCAATACAGTTCCAGCTACAGCTACC 1680

1681 TCCTTGGATAATCTCCAATCCAGCGATTTCGGTTACTTTG 1720

1721 AAAGTGCCAATGCTTTTACATCTTCACTCGGTAACATCGT 1760

1761 GGGTGTTAGAAACTTTAGTGGGACTGCAGGAGTGATTATC 1800

1801 GACAGATTCGAGTTCATTCCAGTTACTGCAACACTCGAGG 1840

1841 CTGAATATAATCTGGAAAGAGCGCAGAAGGCGGTGAATGC 1880

1881 GCTGTTTACGTCTACAAACCAACTAGGGCTAAAAACAAAT 1920

1921 GTAACGGATTATCATATTGATCAAGTGTCCAATTTAGTTA 1960

1961 CGTATTTATCGGATGAATTTTGTCTGGATGAAAAGCGAGA 2000

2001 ATTGTCCGAGAAAGTCAAACATGCGAAGCGACTCAGTGAT 2040

2041 GAACGCAATTTACTCCAAGATTCAAATTTCAAAGACATTA 2080

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2081  ATAGGCAACCAGAACGTGGGTGGGGCGGAAGTACAGGGAT  2120
2121  TACCATCCAAGGAGGGGATGACGTATTTAAAGAAAATTAC  2160
2161  GTCACACTATCAGGTACCTTTGATGAGTGCTATCCAACAT  2200
2201  ATTTGTATCAAAAAATCGATGAATCAAAATTAAAAGCCTT  2240
2241  TACCCGTTATCAATTAAGAGGGTATATCGAAGATAGTCAA  2280
2281  GACTTAGAAATCTATTTAATTCGCTACAATGCAAAACATG  2320
2321  AAACAGTAAATGTGCCAGGTACGGGTTCCTTATGGCCGCT  2360
2361  TTCAGCCCAAAGTCCAATCGGAAAGTGTGGAGAGCCGAAT  2400
2401  CGATGCGCGCCACACCTTGAATGGAATCCTGACTTAGATT  2440
2441  GTTCGTGTAGGGATGGAGAAAAGTGTGCCCATCATTCGCA  2480
2481  TCATTTCTCCTTAGACATTGATGTAGGATGTACAGACTTA  2520
2521  AATGAGGACCTAGGTGTATGGGTGATCTTTAAGATTAAGA  2560
2561  CGCAAGATGGGCACGCAAGACTAGGGAATCTAGAGTTTCT  2600
2601  CGAAGAGAAACCATTAGTAGGAGAAGCGCTAGCTCGTGTG  2640
2641  AAAAGAGCGGAGAAAAAATGGAGAGACAAACGTGAAAAAT  2680
2681  TGGAATGGGAAACAAATATCGTTTATAAAGAGGCAAAAGA  2720
2721  ATCTGTAGATGCTTTATTTGTAAACTCTCAATATGATCAA  2760
2761  TTACAAGCGGATACGAATATTGCCATGATTCATGCGGCAG  2800
2801  ATAAACGTGTTCATAGCATTCGAGAAGCTTATCTGCCTGA  2840
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2841  GCTGTCTGTGATTCCGGGTGTCAATGCGGCTATTTTTGAA  2880

2881  GAATTAGAAGGGCGTATTTTCACTGCATTCTCCCTATATG  2920

2921  ATGCGAGAAATGTCATTAAAAATGGTGATTTTAATAATGG  2960

2961  CTTATCCTGCTGGAACGTGAAAGGGCATGTAGATGTAGAA  3000

3001  GAACAAAACAACCAACGTTCGGTCCTTGTTGTTCCGGAAT  3040

3041  GGGAAGCAGAAGTGTCACAAGAAGTTCGTGTCTGTCCGGG  3080

3081  TCGTGGCTATATCCTTCGTGTCACAGCGTACAAGGAGGGA  3120

3121  TATGGAGAAGGTTGCGTAACCATTCATGAGATCGAGAACA  3160

3161  ATACAGACGAACTGAAGTTTAGCAACTGCGTAGAAGAGGA  3200

3201  AATCTATCCAAATAACACGGTAACGTGTAATGATTATACT  3240

3241  GTAAATCAAGAAGAATACGGAGGTGCGTACACTTCTCGTA  3280

3281  ATCGAGGATATAACGAAGCTCCTTCCGTACCAGCTGATTA  3320

3321  TGCGTCAGTCTATGAAGAAAAATCGTATACAGATGGACGA  3360

3361  AGAGAGAATCCTTGTGAATTTAACAGAGGGTATAGGGATT  3400

3401  ACACGCCACTACCAGTTGGTTATGTGACAAAAGAATTAGA  3440

3441  ATACTTCCCAGAAACCGATAAGGTATGGATTGAGATTGGA  3480

3481  GAAACGGAAGGAACATTTATCGTGGACAGCGTGGAATTAC  3520

3521  TCCTTATGGAGGAA  3534.
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G. einem Struktur-Gen, das für ein insektizides Protein von *B.t.k.* HD-73 in dessen gesamter Länge codiert, mit der Sequenz:

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  1  ATGGACAACAACCCAAACATCAACGAATGCATTCCATACA   40
 41  ACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGGAGA   80
 81  ACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTG  120
121  TCCTTGACACAGTTTCTGCTCAGCGAGTTCGTGCCAGGTG  160
161  CTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTAT  200
201  CTTTGGTCCATCTCAATGGGATGCATTCCTGGTGCAAATT  240
241  GAGCAGTTGATCAACCAGAGGATCGAAGAGTTCGCCAGGA  280
281  ACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTA  320
321  CCAAATCTATGCAGAGAGCTTCAGAGAGTGGGAAGCCGAT  360
361  CCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTCAAT  400
401  TCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATT  440
441  GTTCGCAGTCCAGAACTACCAAGTTCCTCTCTTGTCCGTG  480
481  TACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAG  520
521  ACGTTAGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGC  560
561  AACCATCAATAGCCGTTACAACGACCTTACTAGGCTGATT  600
601  GGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTG  640
641  GCTTGGAGCGTGTCTGGGGTCCTGATTCTAGAGATTGGAT  680
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681   TAGATACAACCAGTTCAGGAGAGAATTGACCCTCACAGTT   720

721   TTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAA   760

761   CCTACCCTATCCGTACAGTGTCCCAACTTACCAGAGAAAT   800

801   CTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTC   840

841   CGTGGTTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCC   880

881   CACACTTGATGGACATCTTGAACAGCATAACTATCTACAC   920

921   CGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAG   960

961   ATCATGGCCTCTCCAGTTGGATTCAGCGGGCCCGAGTTTA   1000

1001  CCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCCACA   1040

1041  ACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGA   1080

1081  ACCTTGTCTTCCACCTTGTACAGAAGACCCTTCAATATCG   1120

1121  GTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGA   1160

1161  GTTCGCCTATGGAACCTCTTCTAACTTGCCATCCGCTGTT   1200

1201  TACAGAAAGAGCGGAACCGTTGATTCCTTGGACGAAATCC   1240

1241  CACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTC   1280

1281  CCACAGGTTGAGCCACGTGTCCATGTTCCGTTCCGGATTC   1320

1321  AGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGTTCT   1360

1361  CTTGGATACACCGTAGTGCTGAGTTCAACAACATCATCGC   1400

1401  ATCCGATAGTATTACTCAAATCCCTGCAGTGAAGGGAAAC   1440
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